TECHNICAL MANUAL

PowerPlex® Fusion System

Instructions for use of Products
DC2402 AND DC2408.

Revised 5/14
TMD039

PowerPlex® Fusion System

All technical literature is available on the Internet at: www.promega.com/protocols/
Please visit the web site to verify that you are using the most current version of this Technical Manual.
Please contact Promega Technical Services if you have questions on use of this system.
E-mail: genetic@promega.com

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1. Description

STR (short tandem repeat) loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction (5–9). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using fluorescence detection following electrophoretic separation.

The PowerPlex® Fusion System[a–g] is a 24-locus multiplex for human identification applications including forensic analysis, relationship testing and research use. This five-color system allows co-amplification and fluorescent detection of the 13 core CODIS (US) loci (CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11), the 12 core European Standard Set loci (TH01, vWA, FGA, D21S11, D3S1358, D8S1179, D18S51, D10S1248, D22S1045, D2S441, D1S1656 and D12S391) and Amelogenin for gender determination. In addition, the male-specific DYS391 locus is included to identify null Y allele results for Amelogenin. The Penta D and Penta E loci are included to increase discrimination and allow searching of databases that include profiles with these Penta loci. Finally, the D2S1338 and D19S433 loci, which are popular loci included in a number of databases, were incorporated to further increase the power of discrimination. This extended panel of STR markers is intended to satisfy both CODIS and ESS recommendations.

The PowerPlex® Fusion System is compatible with the ABI PRISM® 3100 and 3100-*Avan*t Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. Amplification and detection instrumentation may vary. You may need to optimize protocols including amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. In-house validation should be performed.

The PowerPlex® Fusion System provides all materials necessary to amplify STR regions of human genomic DNA, including a hot-start thermostable DNA polymerase, which is a component of the PowerPlex® Fusion 5X Master Mix. This manual contains protocols for use of the PowerPlex® Fusion System with the GeneAmp® PCR System 9700 thermal cycler in addition to protocols to separate amplified products and detect separated material (Figure 1). Protocols to operate the fluorescence-detection instruments should be obtained from the instrument manufacturer.

Information about other Promega fluorescent STR systems is available upon request from Promega or online at: **www.promega.com**

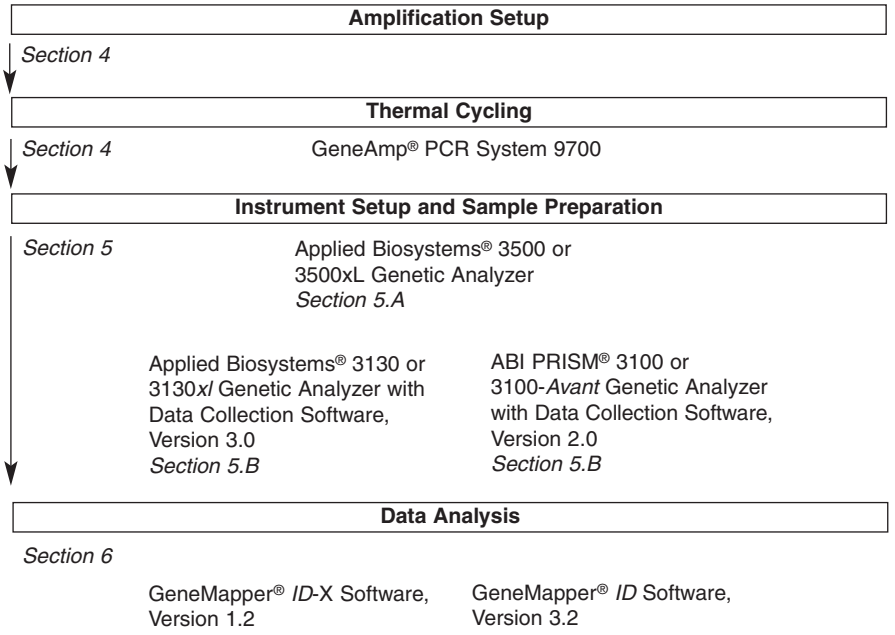
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Figure 1. An overview of the PowerPlex® Fusion System protocol.

2. Product Components and Storage Conditions

Product	Size	Cat.#
PowerPlex® Fusion System	200 reactions	DC2402

Not For Medical Diagnostic Use. This system contains sufficient reagents for 200 reactions of 25µl each. Includes:

Pre-amplification Components Box

1ml	PowerPlex® Fusion 5X Master Mix
1ml	PowerPlex® Fusion 5X Primer Pair Mix
25µl	2800M Control DNA, 10ng/µl
5 × 1,250µl	Water, Amplification Grade

Post-amplification Components Box

100µl	PowerPlex® Fusion Allelic Ladder Mix
2 × 300µl	CC5 Internal Lane Standard 500

Product	Size	Cat.#
PowerPlex® Fusion System	800 reactions	DC2408

Not For Medical Diagnostic Use. This system contains sufficient reagents for 800 reactions of 25µl each. Includes:

Pre-amplification Components Box

4 × 1ml	PowerPlex® Fusion 5X Master Mix
4 × 1ml	PowerPlex® Fusion 5X Primer Pair Mix
25µl	2800M Control DNA, 10ng/µl
10 × 1,250µl	Water, Amplification Grade

Post-amplification Components Box

4 × 100µl	PowerPlex® Fusion Allelic Ladder Mix
8 × 300µl	CC5 Internal Lane Standard 500

The PowerPlex® Fusion Allelic Ladder Mix is provided in a separate, sealed bag for shipping. This component should be moved to the post-amplification box after opening. The Water, Amplification Grade, is provided in a separate, sealed bag for shipping. This component should be moved to the pre-amplification box after opening.

Storage Conditions: For long-term storage, store all components except the 2800M Control DNA at –30°C to –10°C in a nonfrost-free freezer. Store the 2800M Control DNA at 2–10°C. For daily use, the PowerPlex® Fusion System components can be stored for up to 1 week at 2–10°C. The PowerPlex® Fusion 5X Primer Pair Mix, PowerPlex® Fusion Allelic Ladder Mix and CC5 Internal Lane Standard 500 (CC5 ILS 500) are light-sensitive and must be stored in the dark. We strongly recommend that pre-amplification and post-amplification reagents be stored and used separately with different pipettes, tube racks, etc.

Available Separately

The proper panels, bins and stutter text files for use with GeneMapper® *ID* and *ID-X* software are available for download at:
www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/

Matrix standards are required for initial setup of the color separation matrix. The matrix standards are provided separately and are available for ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers (PowerPlex® 5-Dye Matrix Standards, 3100/3130, Cat.# DG4700).

3. Before You Begin

3.A. Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality-control measures that are not contained in this manual (10,11). Guidelines for the validation process are published in the *Internal Validation of STR Systems Reference Manual* (12).

The quality of purified DNA or direct-amplification samples, small changes in buffers, ionic strength, primer concentrations, reaction volume, choice of thermal cycler and thermal cycling conditions can affect PCR success. We suggest strict adherence to recommended procedures for amplification and fluorescence detection. Additional research and validation are required if any modifications to the recommended protocols are made.

PCR-based STR analysis is subject to contamination by very small amounts of human DNA. Extreme care should be taken to avoid cross-contamination when preparing template DNA, handling primer pairs, assembling amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (PowerPlex® Fusion 5X Master Mix, PowerPlex® Fusion 5X Primer Pair Mix, 2800M Control DNA and Water, Amplification Grade) are provided in a separate box and should be stored separately from those used following amplification (PowerPlex® Fusion Allelic Ladder Mix and CC5 Internal Lane Standard 500). Always include a negative control reaction (i.e., no template) to detect reagent contamination. We highly recommend the use of gloves and aerosol-resistant pipette tips.

Some reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

3.B. Spectral Calibration

Proper spectral calibration is critical to evaluate multicolor systems with the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems® 3130, 3130*xl*, 3500 and 3500xL Genetic Analyzers. A matrix must be generated for each individual instrument.

For protocols and additional information on spectral calibration on these instruments, see the *PowerPlex® 5-Dye Matrix Standards, 3100/3130, Technical Bulletin* #TBD024. This manual is available online at: **www.promega.com/protocols/**

4. Protocols for DNA Amplification Using the PowerPlex® Fusion System

The PowerPlex® Fusion System was developed for amplification of extracted DNA and direct-amplification samples. Slight protocol variations are recommended for optimal performance for each template source. Protocols for amplification using extracted DNA (Section 4.A), FTA® and nonFTA storage card punches (Section 4.B) and swabs (Section 4.C) are included in the following amplification sections.

The PowerPlex® Fusion System is optimized for the GeneAmp® PCR System 9700 thermal cycler.

The use of gloves and aerosol-resistant pipette tips is highly recommended to prevent cross-contamination. Keep all pre-amplification and post-amplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup.

Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section 7.

The concentration of 2800M Control DNA was determined by measuring absorbance at 260nm. Quantification of this control DNA by other methods, such as qPCR, may result in a different value. Prepare a fresh DNA dilution for each set of amplifications. Do not store diluted DNA (e.g., 0.25ng/µl or less).

4.A. Amplification of Extracted DNA

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips

We routinely amplify 0.25–0.5ng of template DNA in a 25µl reaction volume using the protocol detailed below.

Amplification Setup

1. Thaw the PowerPlex® Fusion 5X Master Mix, PowerPlex® Fusion 5X Primer Pair Mix and Water, Amplification Grade, completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 5X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

3. Use a clean MicroAmp® plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.

4. Add the final volume of each reagent listed in Table 1 to a sterile tube.

Table 1. PCR Amplification Mix for Amplification of Extracted DNA.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume (µl)
Water, Amplification Grade[1]	to a final volume of 25.0µl	×		=	
PowerPlex® Fusion 5X Master Mix	5.0µl	×		=	
PowerPlex® Fusion 5X Primer Pair Mix	5.0µl	×		=	
template DNA (0.25–0.5ng)[2,3]	up to 15µl				
total reaction volume	25µl				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® Fusion 5X Master Mix and PowerPlex® Fusion 5X Primer Pair Mix. The template DNA will be added at Step 6.

[2]Store DNA templates in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE^{-4} buffer with 20µg/ml glycogen. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the volume of DNA added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA) or other PCR inhibitors, which may be present at low concentrations depending on the source of the template DNA and the extraction procedure used.

[3]Apparent DNA concentrations can differ, depending on the DNA quantification method used (13). The amount of DNA template recommended here is based on DNA concentrations determined by measuring absorbance at 260nm. We strongly recommend that you perform experiments to determine the optimal DNA amount based on your DNA quantification method.

4.A. Amplification of Extracted DNA (continued)

5. Vortex the PCR amplification mix for 5–10 seconds, then add the PCR amplification mix to each reaction well.

   Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.

6. Add the template DNA (0.25–0.5ng) for each sample to the respective well containing PCR amplification mix.

7. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute an aliquot to 0.5ng in the desired template DNA volume. Add 0.5ng of diluted DNA to a reaction well containing PCR amplification mix.

8. For the negative amplification control, pipet Water, Amplification Grade, or TE^{-4} buffer instead of template DNA into a reaction well containing PCR amplification mix.

9. Seal the plate, or close the tubes. **Optional:** Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

Amplification and detection instrumentation may vary. You may need to optimize protocols including the amount of template DNA, cycle number, injection conditions and loading volume for your laboratory instrumentation. Testing at Promega shows that 30 cycles works well for 0.5ng of purified DNA templates.

1. Place the MicroAmp® plate or reaction tubes in the thermal cycler.

2. Select and run the recommended protocol. Be sure that Max mode is selected as the ramp speed. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below. The estimated total cycling time is 1.5 hours.

Thermal Cycling Protocol[1]
96°C for 1 minute, then:
94°C for 10 seconds 59°C for 1 minute 72°C for 30 seconds for 30 cycles, then:
60°C for 10 minutes
4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with Max mode as the ramp speed. (This requires a silver or gold-plated silver sample block). The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select "Max" for the ramp speed, and enter the reaction volume.

3. After completion of the thermal cycling protocol, store amplified samples at –20°C in a light-protected box.

   Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

4.B. Direct Amplification of DNA from Storage Card Punches

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips
- PunchSolution™ Kit (Cat.# DC9271) for nonFTA card punches
- 1.2mm Harris Micro-Punch or equivalent manual punch and cutting mat or automated punch system

This section contains a protocol for direct amplification of DNA from storage card punches using the PowerPlex® Fusion System and GeneAmp® PCR System 9700 thermal cycler.

We recommend amplifying one or two 1.2mm punches of a storage card containing a buccal sample or one 1.2mm punch of a storage card containing whole blood in a 25µl reaction volume using the protocols detailed below. The PowerPlex® Fusion System is optimized for the GeneAmp® PCR System 9700 thermal cycler.

Note: You will need to optimize and validate the number of storage card punches per reaction in your laboratory. See the PCR optimization recommendations at the end of the section.

FTA®-based sample types include:

- Buccal cells collected on FTA® cards with Whatman EasiCollect™ or Fitzco Sampact™ devices
- Buccal cells collected with sterile swabs transferred to FTA® or Indicating FTA® cards
- Liquid blood (from collection or storage Vacutainer® tubes or finger sticks) spotted onto FTA® cards

NonFTA sample types include:

- Buccal samples on Bode Buccal DNA Collector™ devices
- Blood and buccal samples on nonFTA card punches (e.g., S&S 903)

Pretreat nonFTA sample types with the PunchSolution™ Kit (Cat.# DC9271) to lyse nonFTA samples before adding the PCR amplification mix. For more information, see the *PunchSolution™ Kit Technical Manual* #TMD038. Failure to pretreat these samples may result in incomplete profiles.

Use a manual punch tool with a 1.2mm tip to manually create sample disks from a storage card. Place tip near the center of the sample spot, and with a twisting or pressing action, cut a 1.2mm sample disk. Use the plunger to eject the disk into the appropriate well of a reaction plate.

4.B Direct Amplification of DNA from Storage Card Punches (continued)

Automated punchers also can be used to create sample disks. Refer to the user's guide for your instrument for assistance with generating 1.2mm disks, technical advice and troubleshooting information.

Note: Static may be problematic when adding a punch to a well. For FTA® card punches, adding PCR amplification mix to the well before adding the punch may help alleviate static problems. For nonFTA card punches, adding PunchSolution™ Reagent to the well before adding the punch during pretreatment may help alleviate static problems.

Amplification Setup

1. Thaw the PowerPlex® Fusion 5X Master Mix, PowerPlex® Fusion 5X Primer Pair Mix and Water, Amplification Grade, completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 5X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

3. Use a clean MicroAmp® plate for reaction assembly, and label appropriately. Alternatively, determine the number of clean, 0.2ml reaction tubes required, and label appropriately.

4. Add the final volume of each reagent listed in Table 2 to a sterile tube.

Table 2. PCR Amplification Mix for Direct Amplification of DNA from Storage Card Punches.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	15µl	×		=	
PowerPlex® Fusion 5X Master Mix	5.0µl	×		=	
PowerPlex® Fusion 5X Primer Pair Mix	5.0µl	×		=	
total reaction volume	25µl				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® Fusion 5X Master Mix and PowerPlex® Fusion 5X Primer Pair Mix. For FTA® card punches, the template DNA will be aded at Step 6.

5. Vortex the PCR amplification mix for 5–10 seconds, then pipet 25µl of PCR amplification mix into each reaction well.

Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.

6. For FTA® storage cards, add one or two 1.2mm punches from a card containing a buccal sample or one 1.2mm punch from a card containing whole blood to the appropriate wells of the reaction plate. For nonFTA card punches, add the PCR amplification mix to the PunchSolution™ Reagent-treated punches.

   Note: It also is acceptable to add the FTA® card punch first, then add the PCR amplification mix.

7. For the positive amplification control, add 1µl of 2800M Control DNA (10ng) to a reaction well containing 25µl of PCR amplification mix.

   Notes:

   1. Do not include blank storage card punches in the positive control reactions.
   2. Optimization of the amount of control DNA may be required, depending on cycling conditions and laboratory preferences.

8. Reserve a well containing PCR amplification mix as a negative amplification control.

   Note: An additional negative control with a blank punch may be performed to detect contamination from the storage card or punch device.

9. Seal the plate, and briefly centrifuge the plate to bring storage card punches to the bottom of the wells.

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the number of storage card punches, cycle number (25–28 cycles), injection time and loading volume for your laboratory instrumentation. Testing at Promega shows that 27 cycles works well for a variety of sample types. Buccal samples may require more amplification cycles than blood samples. Cycle number should be optimized in each laboratory for each sample type that is amplified.

1. Place the MicroAmp® plate in the thermal cycler.

4.B Direct Amplification of DNA from Storage Card Punches (continued)

2. Select and run the recommended protocol. Be sure that Max mode is selected as the ramp speed. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below. The estimated total cycle time is 1.5 hours.

Thermal Cycling Protocol[1]
96°C for 1 minute, then:
94°C for 10 seconds 59°C for 1 minute 72°C for 30 seconds for 27 cycles, then:
60°C for 20 minutes
4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with Max mode as the ramp speed. (This requires a silver or gold-plated silver sample block.) The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select "Max" for the ramp speed, and enter the reaction volume.

Note: The final extension for direct amplification was extended to 20 minutes compared to 10 minutes for the extracted DNA protocol to allow sufficient time for adenylation of large amounts of amplicon.

3. After completion of the thermal cycling protocol, store amplified samples at –20°C in a light-protected box.

   Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

PCR Optimization

Cycle number should be optimized based on the results of an initial experiment to determine the sensitivity with your collection method, sample types, number of punches and instrumentation.

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Depending on your preferred protocol, place one or two 1.2mm storage card punches containing a buccal sample or one 1.2mm punch of a storage card containing whole blood in each well of a reaction plate. Be sure to pretreat nonFTA samples with the PunchSolution™ Kit (Cat.# DC9271).
3. Prepare four identical reaction plates with punches from the same samples.
4. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (25–28 cycles).
5. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type and number of storage card punches.

4.C. Direct Amplification of DNA from Swabs

Materials to Be Supplied by the User

- GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems)
- microcentrifuge
- MicroAmp® optical 96-well reaction plate or 0.2ml MicroAmp® reaction tubes (Applied Biosystems)
- aerosol-resistant pipette tips
- SwabSolution™ Kit (Cat.# DC8271)

This section contains a protocol for amplifying DNA from swab extracts using the PowerPlex® Fusion System and GeneAmp® PCR System 9700 thermal cycler.

Pretreat OmniSwab™ (GE Healthcare) or cotton swabs with the SwabSolution™ Kit (Cat.# DC8271) as described in the *SwabSolution™ Kit Technical Manual* #TMD037 to generate a swab extract.

Amplification Setup

1. Thaw the PowerPlex® Fusion 5X Master Mix, PowerPlex® Fusion 5X Primer Pair Mix and Water, Amplification Grade, completely.

   Note: Centrifuge tubes briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the 5X Primer Pair Mix or 5X Master Mix after vortexing, as this may cause the reagents to be concentrated at the bottom of the tube.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does consume a small amount of each reagent, it ensures that you will have enough PCR amplification mix for all samples. It also ensures that each reaction contains the same PCR amplification mix.

3. Use a clean MicroAmp® plate for reaction assembly, and label appropriately.

4. Add the final volume of each reagent listed in Table 3 to a sterile tube.

Table 3. PCR Amplification Mix for Direct Amplification of DNA from Swabs.

PCR Amplification Mix Component[1]	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade	13µl	×		=	
PowerPlex® Fusion 5X Master Mix	5.0µl	×		=	
PowerPlex® Fusion 5X Primer Pair Mix	5.0µl	×		=	
swab extract	2.0µl				
total reaction volume	25µl				

[1]Add Water, Amplification Grade, to the tube first, then add PowerPlex® Fusion 5X Master Mix and PowerPlex® Fusion 5X Primer Pair Mix. The swab extract will be added at Step 6.

4.C. Direct Amplification of DNA from Swabs (continued)

5. Vortex the PCR amplification mix for 5–10 seconds, then pipet 23µl of PCR amplification mix into each reaction well.

Failure to vortex the PCR amplification mix sufficiently can result in poor amplification or locus-to-locus imbalance.

6. Pipet 2.0µl of swab extract for each sample into the appropriate well of the reaction plate.

7. For the positive amplification control, vortex the tube of 2800M Control DNA, then dilute an aliquot to 5.0ng/µl. Add 2µl (10ng) to a reaction well containing 23µl of PCR amplification mix.

8. For the negative amplification control, pipet 2.0µl of Water, Amplification Grade, or TE^{-4} buffer instead of swab extract into a reaction well containing PCR amplification mix.

9. Seal the plate. **Optional:** Briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles.

Thermal Cycling

Amplification and detection instrumentation may vary. You will need to optimize protocols including the amount of template DNA, cycle number (25–28 cycles), injection time and loading volume for your laboratory instrumentation. Testing at Promega shows that 27 cycles works well for a variety of sample types. Cycle number will need to be optimized in each laboratory for each sample type that is amplified.

1. Place the MicroAmp® plate in the thermal cycler.

2. Select and run the recommended protocol. Be sure that Max mode is selected as the ramp speed. The preferred protocol for use with the GeneAmp® PCR System 9700 thermal cycler is provided below. The estimated total cycle time is 1.5 hours.

Thermal Cycling Protocol[1]
96°C for 1 minute, then:
94°C for 10 seconds 59°C for 1 minute 72°C for 30 seconds for 27 cycles, then:
60°C for 20 minutes
4°C soak

[1]When using the GeneAmp® PCR System 9700 thermal cycler, the program must be run with Max mode as the ramp speed. (This requires a silver or gold-plated silver sample block.) The ramp speed is set after the thermal cycling run is started. The Select Method Options screen appears. Select "Max" for the ramp speed, and enter the reaction volume.

Note: The final extension for direct amplification was extended to 20 minutes compared to 10 minutes for the extracted DNA protocol to allow sufficient time for adenylation of large amounts of amplicon.

3. After completion of the thermal cycling protocol, store amplified samples at –20°C in a light-protected box.

   Note: Long-term storage of amplified samples at 4°C or higher may produce artifacts.

PCR Optimization

Cycle number should be optimized based on the results of an initial experiment to determine the sensitivity with your collection method, sample types and instrumentation.

1. Choose several samples that represent typical sample types you encounter in the laboratory. Prepare them as you would using your normal workflow.
2. Prepare four identical reaction plates with aliquots of the same swab extracts.
3. Amplify samples using the thermal cycling protocol provided above, but subject each plate to a different cycle number (25–28 cycles).
4. Following amplification, use your laboratory's validated separation and detection protocols to determine the optimal cycle number for the sample type.

5. Instrument Setup and Sample Preparation

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- 96-well retainer & base set (standard) (Applied Biosystems Cat.# 4410228)
- POP-4® polymer for the Applied Biosystems® 3500 or 3500xL Genetic Analyzer
- anode buffer container
- cathode buffer container
- MicroAmp® optical 96-well plate and septa, or equivalent
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

Sample Preparation

1. Thaw the CC5 Internal Lane Standard 500.

   Note: Centrifuge tube briefly to bring contents to the bottom, then vortex for 15 seconds before each use. Do not centrifuge after vortexing, as this may cause the size standard to be concentrated at the bottom of the tube.

2. Prepare a loading cocktail by combining and mixing CC5 Internal Lane Standard 500 and Hi-Di™ formamide as follows:

   [(1.0µl CC5 ILS 500) × (# samples)] + [(10.0µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks based on laboratory preferences. Keep the volume of formamide at 10.0µl per well, and adjust the volume added to the wells in Step 4 accordingly.

3. Vortex for 10–15 seconds to mix.

4. Pipet 11µl of formamide/internal lane standard mix into each well.

5. Add 1µl of amplified sample (or 1µl of PowerPlex® Fusion Allelic Ladder Mix). Cover wells with appropriate septa.

   Note: Instrument detection limits vary; therefore, injection time, injection voltage or the amount of sample mixed with loading cocktail may need to be increased or decreased. To modify the injection time or injection voltage in the run module, select "Instrument Protocol" from the Library menu in the data collection software. If peak heights are higher than desired, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program to achieve the desired signal intensity. If the injection time or voltage is reduced, a decreased peak amplitude threshold for the orange channel may be required for proper sizing.

6. Centrifuge plate briefly to remove air bubbles from the wells.

7. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

Instrument Preparation

Refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* for the instrument maintenance schedule and instructions to install the capillary array, buffers and polymer pouch and perform a spatial calibration. Samples may be analyzed as described in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*.

1. Open the 3500 Data Collection Software. The Dashboard screen will launch (Figure 2). Ensure that the Consumables Information and Maintenance Notifications are acceptable.

   Set the oven temperature to 60°C, then select "Start Pre-Heat" at least 30 minutes prior to the first injection to preheat the oven.

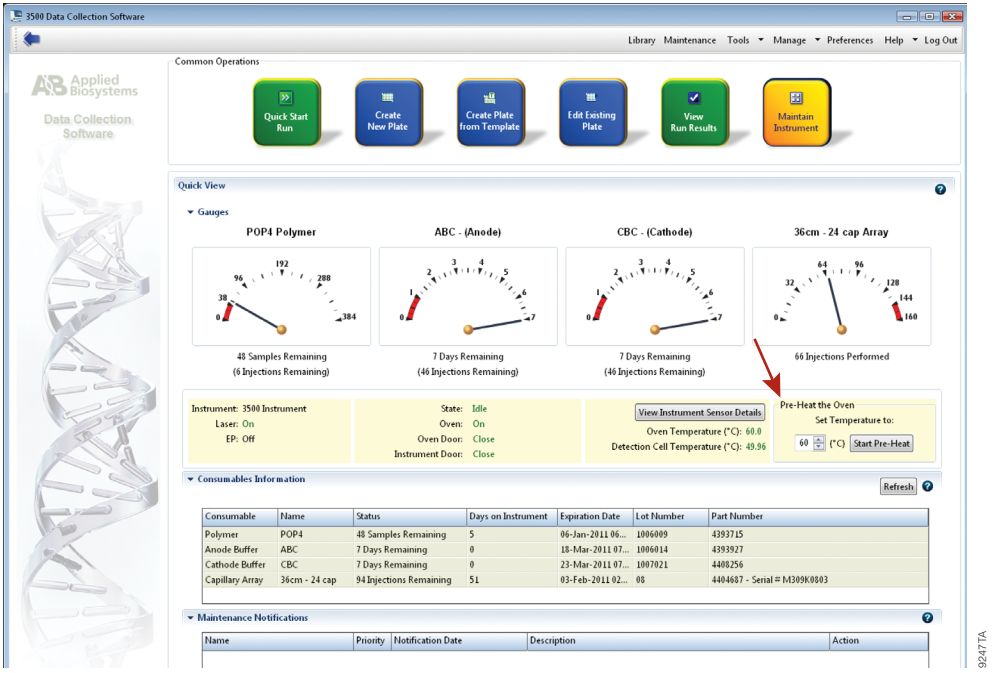

Figure 2. The Dashboard.

2. To create a new Instrument Protocol, navigate to the Library, select "Instrument Protocol", then select "Create". Alternatively, a previously created Instrument Protocol may be used.

5.A. Detection of Amplified Fragments Using the Applied Biosystems® 3500 or 3500xL Genetic Analyzer (continued)

Figure 3 shows the settings used at Promega for the Applied Biosystems® 3500xL Genetic Analyzer for the application type, dye set, capillary length, polymer, run module and appropriate protocol information. The only setting that was changed from the default settings is dye set.

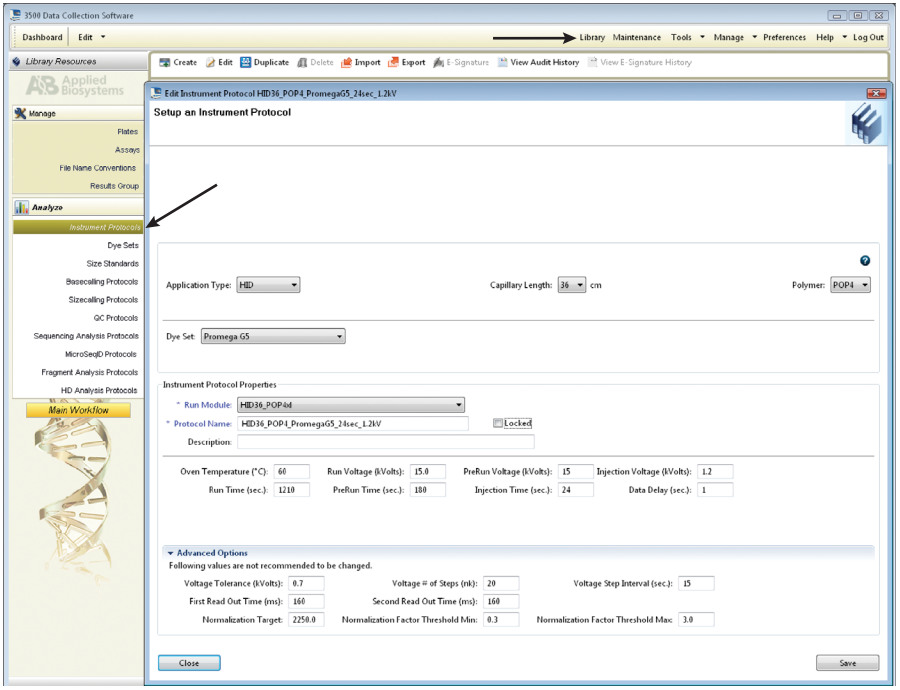

Figure 3. The Create New Instrument Protocol window.

The recommended settings are:

Application Type	HID
Capillary Length	36cm
Polymer	POP-4®
Dye Set	G5 (Promega G5 spectral)
Run Module	HID36_POP4(xl)
Injection Time[1]	24 seconds
Injection Voltage	1.2kV
Run Time	1,210–1,500 seconds

[1]Injection time may be modified (2–24 seconds) to increase or decrease peak heights.

When creating an Instrument Protocol, be sure to select the same dye set that was used to perform the Promega 5-dye spectral calibration. We recommend using a run time of 1,210–1,500 seconds and the default injection conditions.

Run time and other instrument settings should be optimized and validated in your laboratory.

When optimizing injection conditions in your laboratory, you may choose to create specific Instrument Protocols for each condition tested. If a single Instrument Protocol is used, follow the instructions in the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide* to edit a library entry.

Assign a descriptive protocol name.

Note: For more detailed information refer to the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

3. To create a new Size Standard for the QC protocol, navigate to the Library. Select "Size Standards", then select "Create". Alternatively, a previously created Size Standard may be used.

   Assign the size standard the name "ILS500" or another appropriate name. Choose "Orange" as the Dye Color. The fragments in the size standard are 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases. See Figure 4.

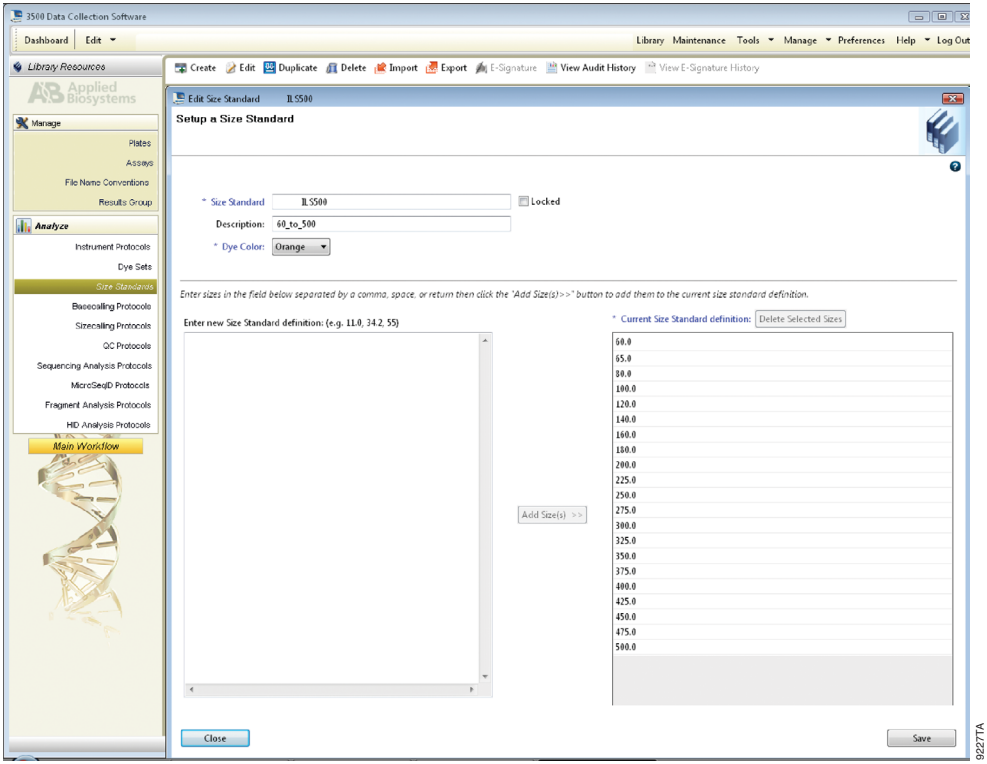

Figure 4. The Create New Size Standard window.

4. To create a new QC Protocol, navigate to the Library. Select "QC Protocols", then select "Create". Alternatively, a previously created QC Protocol may be used.

   Assign a descriptive protocol name. Select the size standard created in Step 3. The settings for the QC protocol should be based on the internally validated conditions for the PowerPlex® Fusion System on the Applied Biosystems® 3500 or 3500xL Genetic Analyzer. Figure 5 shows one option for these settings.

   Note: Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes.

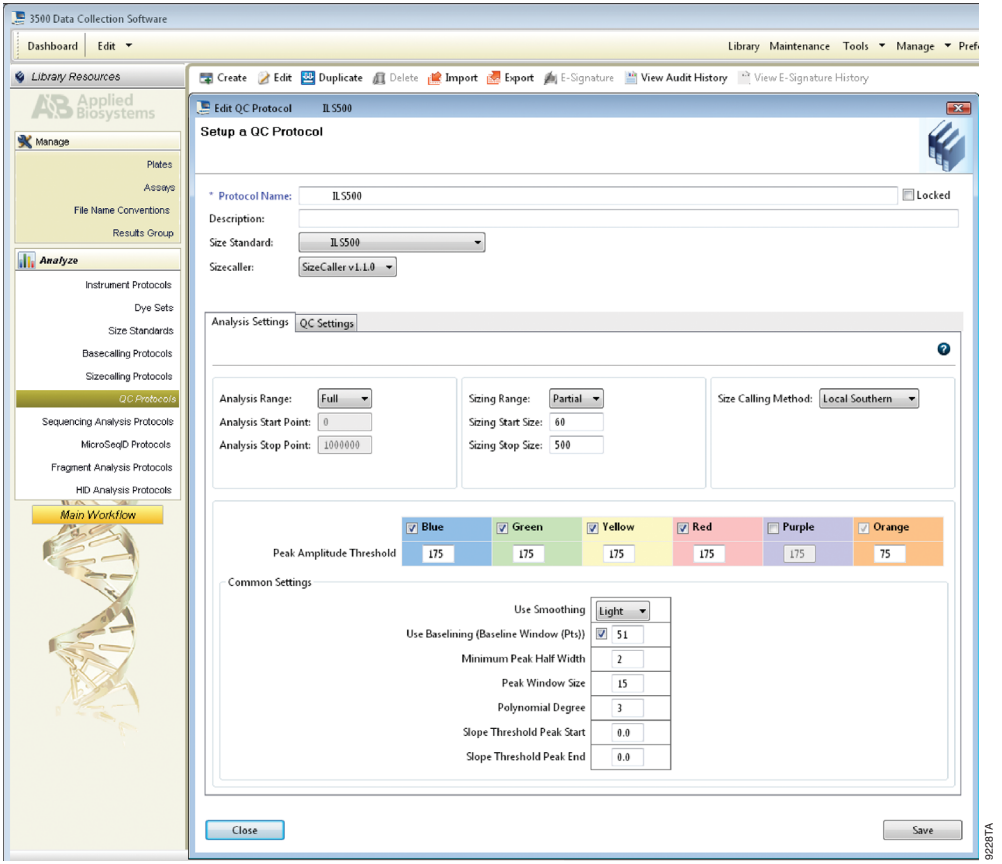

Figure 5. The Create New QC Protocol window.

5. To create a new Assay, navigate to the Library. Select "Assays", then select "Create". Alternatively, a previously created Assay may be used.

   In the Create New Assay window (Figure 6), select the Instrument Protocol created in Step 2 and the QC Protocol created in Step 4. Assign a descriptive assay name. Select the application type "HID". An Assay is required for all named samples on a plate.

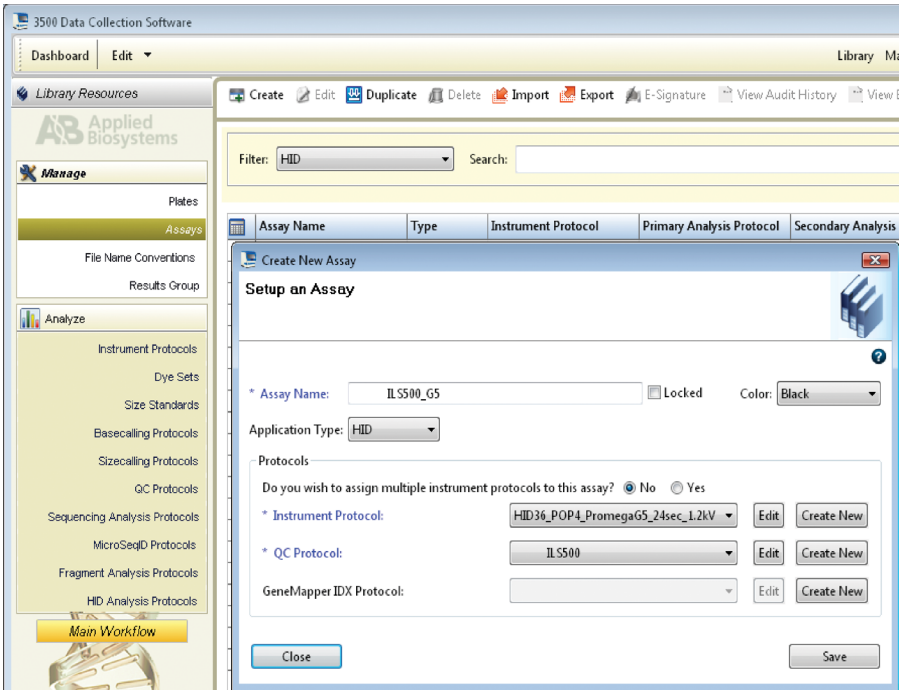

Figure 6. The Create New Assay window.

6. To create a new File Name Convention (Figure 7), navigate to the Library. Select "File Name Conventions", then select "Create". Alternatively, a previously created File Name Convention may be used.

   Select the File Name Attributes according to laboratory practices, and save with a descriptive name.

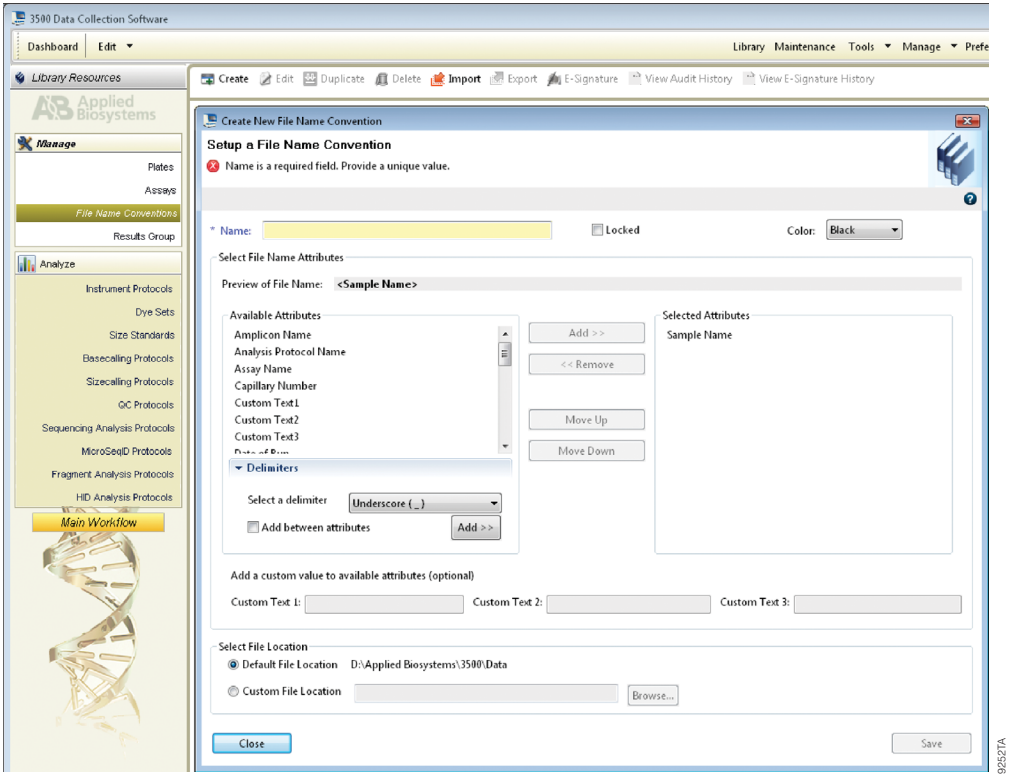

Figure 7. The Create New File Name Convention window.

7. To create a new Results Group (Figure 8), navigate to the Library. Select "Results Group", then select "Create". Alternatively, a previously created Results Group may be used.

   Select the Results Group Attributes according to laboratory practices. Save with a descriptive name.

8. To create a New Plate, navigate to the Library, and from the Manage menu, select "Plates", then "Create".

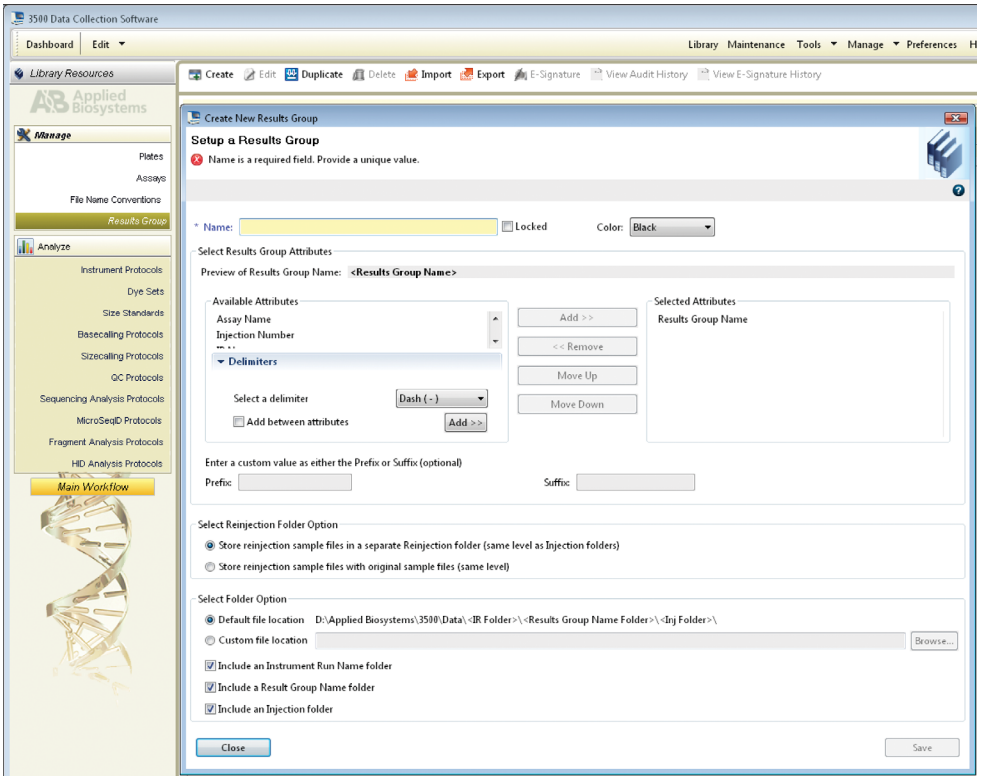

Figure 8. The Create New Results Group window.

9. Assign a descriptive plate name. Select the plate type "HID" from the drop-down menu (Figure 9).

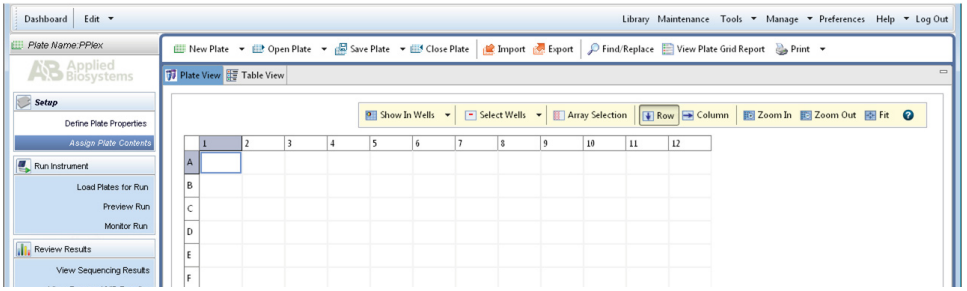

Figure 9. Defining plate properties.

10. Select "Assign Plate Contents" (Figure 10).
11. Assign sample names to wells.
12. In the lower left portion of the screen, under "Assays", use the Add from Library option to select the Assay created in Step 5 or one previously created. Click on the Add to Plate button, and close the window.

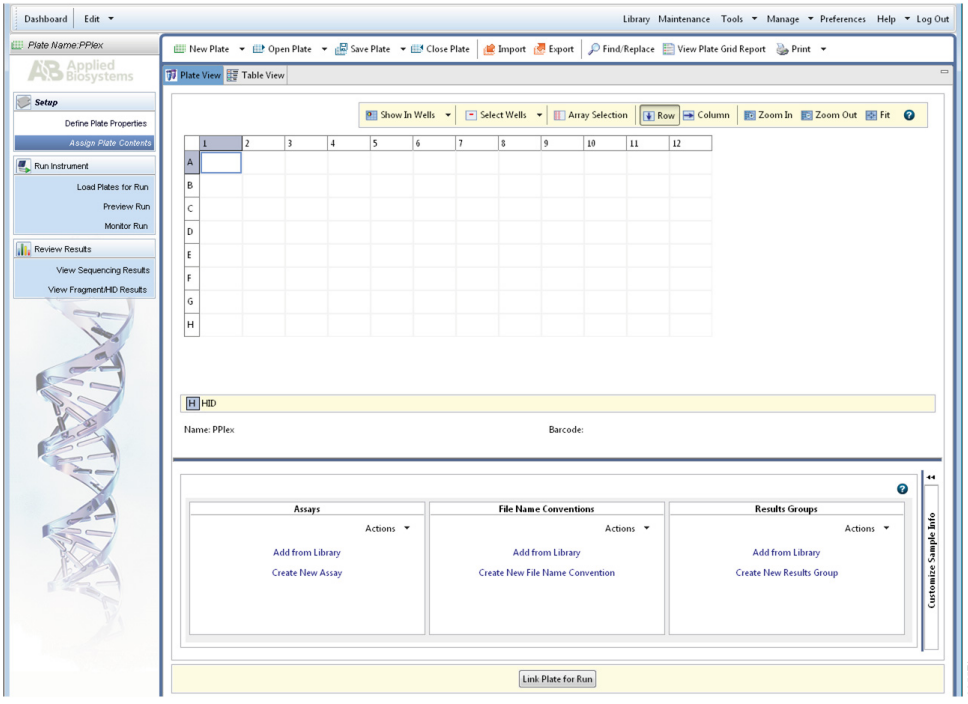

Figure 10. Assigning plate contents.

13. Under "File Name Convention", use the Add from Library option to select the File Name Convention created in Step 6 or one previously created. Click on the Add to Plate button, and close the window.

14. Under "Results Groups", use the Add from Library option to select the Results Group created in Step 7 or one previously created. Click on the Add to Plate button, and close the window.

15. Highlight the sample wells, then select the boxes in the Assays, File Name Conventions and Results Groups that pertain to those samples.

16. Select "Link Plate for Run".

17. The Load Plate window will appear. Select "Yes".

18. In the Run Information window (Figure 11), assign a Run Name. Select "Start Run" (not shown).

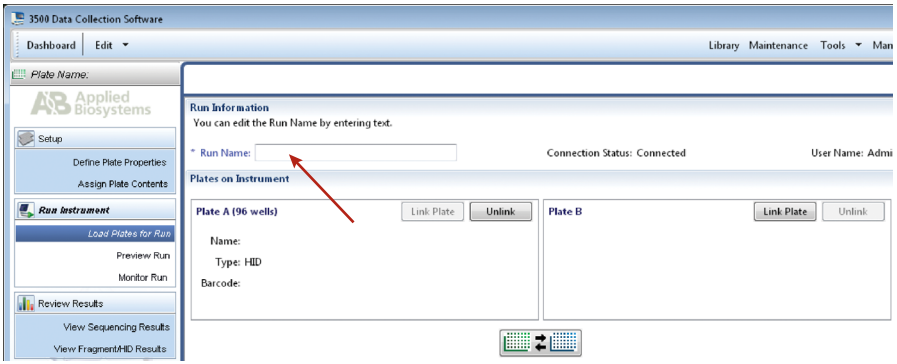

Figure 11. Assigning a run name.

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3100 or 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4®) for the 3100 or 3130
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate and septa, or equivalent
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

Sample Preparation

1. Thaw the CC5 Internal Lane Standard 500.

   Note: Centrifuge tube briefly to bring contents to the bottom, then vortex for 15 seconds before each use. Do not centrifuge after vortexing, as this may cause the size standard to be concentrated at the bottom of the tube.

2. Prepare a loading cocktail by combining and mixing CC5 Internal Lane Standard 500 and Hi-Di™ formamide as follows:

   [(1.0µl CC5 ILS 500) × (# samples)] + [(10.0µl Hi-Di™ formamide) × (# samples)]

   Note: The volume of internal lane standard used in the loading cocktail can be increased or decreased to adjust the intensity of the size standard peaks based on laboratory preferences. Keep the volume of formamide at 10.0µl per well, and adjust the volume added to the wells in Step 4 accordingly.

3. Vortex for 10–15 seconds to mix.

4. Pipet 11µl of formamide/internal lane standard mix into each well.

5. Add 1µl of amplified sample (or 1µl of PowerPlex® Fusion Allelic Ladder Mix). Cover wells with appropriate septa.

   Note: Instrument detection limits vary; therefore, injection time, injection voltage or the amount of sample mixed with loading cocktail may need to be adjusted. Use the Module Manager in the data collection software to modify the injection time or voltage in the run module (see Instrument Preparation below). If the injection time or voltage is reduced, a decreased peak amplitude threshold for the orange channel may be required for proper sizing.

6. Centrifuge plate briefly to remove air bubbles from the wells.

7. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

Instrument Preparation

Refer to the instrument user's manual for instructions on cleaning, installing the capillary array, performing a spatial calibration and adding polymer.

Analyze samples as described in the user's manual for the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, and the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0, with the following exceptions.

1. In the Module Manager, select "New". Select "Regular" in the Type drop-down list, and select "HIDFragmentAnalysis36_POP4" in the Template drop-down list. Confirm that the injection time is 5 seconds, the injection voltage is 3kV and the run time is 1,500 seconds. Give a descriptive name to your run module, and select "OK".

   Note: Instrument sensitivities can vary. The injection time and voltage may be adjusted in the Module Manager. A suggested range for the injection time is 3–22 seconds and for the injection voltage is 1–3kV.

2. In the Protocol Manager, select "New". Type a name for your protocol. Select "Regular" in the Type drop-down list, and select the run module you created in the previous step in the Run Module drop-down list. Lastly, select "G5" in the dye-set drop-down list. Select "OK".

3. In the Plate Manager, create a new plate record as described in the instrument user's manual. In the dialog box that appears, select "GeneMapper—Generic" in the Application drop-down list, and select the appropriate plate type (96-well). Add entries in the owner and operator windows, and select "OK".

   Note: If autoanalysis of sample data is desired, refer to the instrument user's manual for instructions.

5.B. Detection of Amplified Fragments Using the ABI PRISM® 3100 or 3100-*Avant* Genetic Analyzer with Data Collection Software, Version 2.0, or the Applied Biosystems® 3130 or 3130*xl* Genetic Analyzer with Data Collection Software, Version 3.0 (continued)

4. In the GeneMapper plate record, enter sample names in the appropriate cells. Scroll to the right. In the Results Group 1 column, select the desired results group. In the Instrument Protocol 1 column, select the protocol you created in Step 2. Be sure this information is present for each row that contains a sample name. Select "OK".

   Note: To create a new results group, select "New" in the drop-down menu in the Results Group column. Select the General tab, and enter a name. Select the Analysis tab, and select "GeneMapper—Generic" in the Analysis type drop-down list.

5. Place samples in the instrument, and close the instrument doors.

6. In the spectral viewer, select dye set G5, and confirm that the active dye set is the file generated for the PowerPlex® 5-dye chemistry.

   It is critical to select the correct G5 spectral for the PowerPlex® 5-dye chemistry.

   If the PowerPlex® 5-dye chemistry is not the active dye set, locate the PowerPlex® 5-dye spectral in the List of Calibrations for Dye Set G5, and select "Set".

7. In the run scheduler, locate the plate record that you just created in Steps 3 and 4, and click once on the name to highlight it.

8. Once the plate record is highlighted, click the plate graphic that corresponds to the plate on the autosampler that contains your amplified samples.

9. When the plate record is linked to the plate, the plate graphic will change from yellow to green, and the green Run Instrument arrow becomes enabled.

10. Click on the green Run Instrument arrow on the toolbar to start the sample run.

11. Monitor electrophoresis by observing the run, view, array or capillaries viewer window in the data collection software. Each injection will take approximately 40 minutes.

6. Data Analysis

6.A. Importing PowerPlex® Fusion Panels, Bins and Stutter Text Files with GeneMapper® *ID*-X Software, Version 1.2

To facilitate analysis of data generated with the PowerPlex® Fusion System, we have created panels and bins text files to allow automatic assignment of genotypes using GeneMapper® *ID*-X software. We recommend that users receive training from Applied Biosystems on the GeneMapper® *ID*-X software to familiarize themselves with proper operation of the software.

Note: The panels, bins and stutter text files mentioned here are compatible with earlier versions of the GeneMapper® *ID*-X software.

Getting Started

1. To obtain the proper panels, bins and stutter text files for the PowerPlex® Fusion System go to: **www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/**
2. Enter your contact information, and select "GeneMapper ID-X". Select "Submit".
3. Save the PowerPlex_Fusion_Panels_IDX_vX.x.txt, PowerPlex_Fusion_Bins_IDX_vX.x.txt and PowerPlex_Fusion_Stutter_IDX_vX.x.txt files, where "X.x" refers to the most recent version of the panels, bins and stutter text files, to a known location on your computer.

Importing Panels, Bins and Stutter Text Files

1. Open the GeneMapper® *ID*-X software.
2. Select "Tools", then "Panel Manager".
3. Highlight the Panel Manager icon in the upper left navigation pane.
4. Select "File", then "Import Panels".
5. Navigate to the panels text file imported in the Getting Started Section. Select the file, then "Import".
6. In the navigation pane, highlight the PowerPlex Fusion panels folder that you just imported in Step 5.
7. Select "File", then "Import Bin Set".
8. Navigate to the bins text file imported in the Getting Started Section. Select the file, then "Import".
9. In the navigation pane, highlight the PowerPlex Fusion panels folder that you just imported in Step 5.
10. Select "File", then "Import Marker Stutter". A warning box will appear asking if you want to overwrite current values. Select "Yes".

6.A. Importing PowerPlex® Fusion Panels, Bins and Stutter Text Files with GeneMapper® *ID*-X Software, Version 1.2 (continued)

11. Navigate to the stutter text file imported in the Getting Started Section. Select the file, then "Import".
12. At the bottom of the Panel Manager window, select "OK". This will save the panels, bins and stutter text files and close the window.

6.B. Importing the CC5 ILS 500 IDX Size Standard into GeneMapper® *ID*-X Software, Version 1.2

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.C.

The CC5_ILS_500_IDX.xml file is available for download at: **www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/**

Save the CC5_ILS_500_IDX.xml file to a known location on your computer.

1. Select "Tools", then "GeneMapper ID-X Manager".
2. Select the Size Standard tab.
3. Select "Import".
4. Navigate to the location of the CC5_ILS_500_IDX.xml file on your computer.
5. Highlight the file, then select "Import".
6. Select "Done" to save changes and close the GeneMapper® *ID*-X Manager.

6.C. Creating a Size Standard with GeneMapper® *ID*-X Software, Version 1.2

1. Select "Tools", then "GeneMapper ID-X Manager".
2. Select the Size Standard tab.
3. Select "New".
4. In the Size Standard Editor window (Figure 12), select "GeneMapper ID-X Security Group" as the Security Group. This allows access for all users of the software. Other security groups may be used.
5. Enter a detailed name, such as "CC5_ILS_500_IDX".
6. Choose "Orange" for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 9.C, Figure 24.
8. Select "OK".

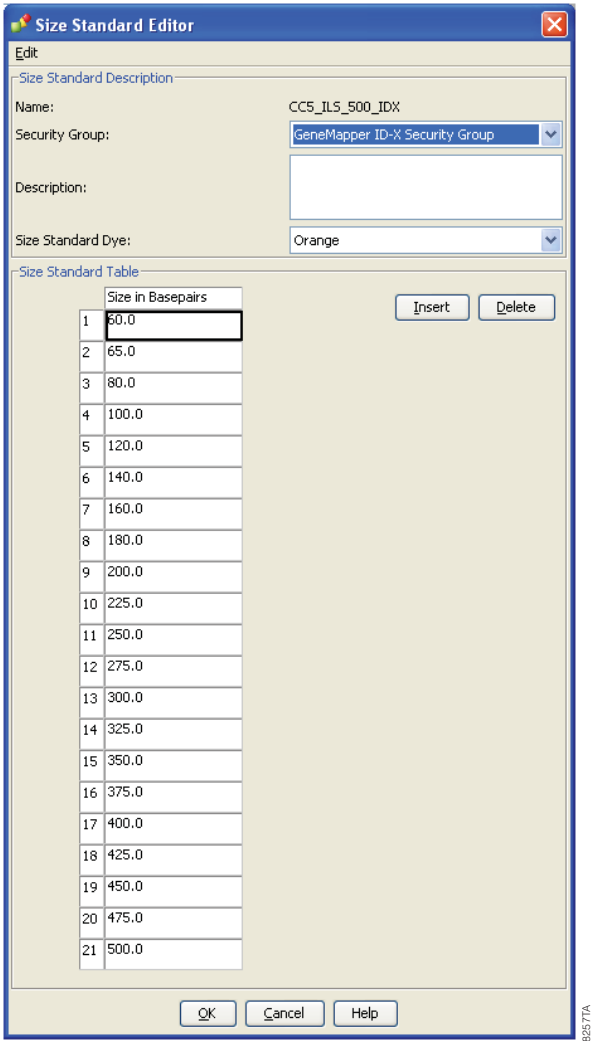

Figure 12. The GeneMapper® *ID*-X Size Standard Editor.

6.D. Creating a Casework Analysis Method with GeneMapper® *ID-X* Software, Version 1.2

These instructions are intended as a guide to start analyzing data in GeneMapper® *ID*-X software. They are not intended as a comprehensive guide for using GeneMapper® *ID*-X software. We recommend that users contact Applied Biosystems for training on the software.

1. Select "Tools", then "GeneMapper ID-X Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.

6.D. Creating a Casework Analysis Method with GeneMapper® *ID-X* Software, Version 1.2 (continued)

4. In the Analysis Method Editor window, select "GeneMapper ID-X Security Group" as the Security Group. This allows access to all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as "PowerPlex Fusion".
6. Select the Allele tab (Figure 13).
7. Select the bins text file that was imported in Section 6.A.
8. Ensure that the "Use marker-specific stutter ratio and distance if available" box is checked.
9. We recommend the values shown in Figure 13 for proper filtering of stutter peaks when using the PowerPlex® Fusion System. You may need to optimize these settings. In-house validation should be performed.

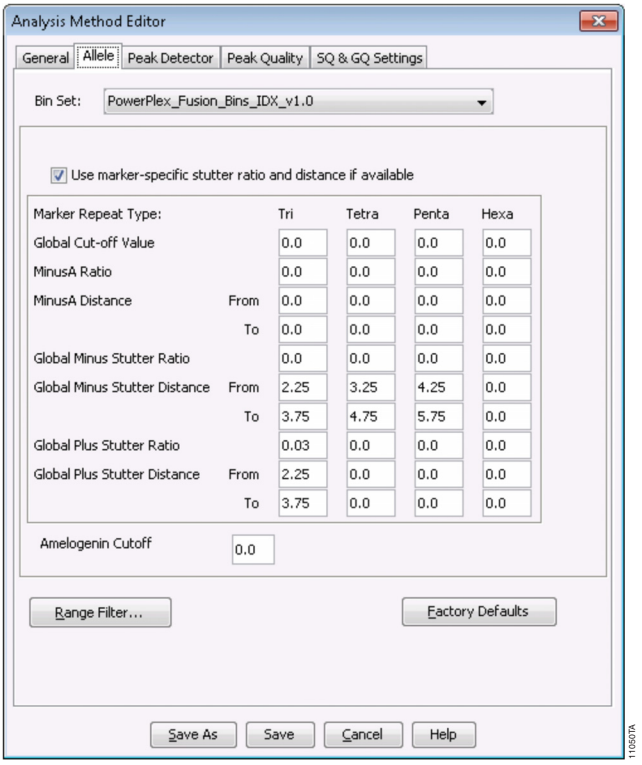

Figure 13. The GeneMapper® *ID-X* Allele tab.

10. Select the Peak Detector tab. Figure 14 shows an example of settings used at Promega. You may need to optimize these settings. In-house validation should be performed.

Notes:

1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.
2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds are usually 50–150RFU for data generated on the ABI PRISM® 3100 and 3100-*Avan*t Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers. For the Applied Biosystems® 3500 and 3500xL Genetic Analyzers, Life Technologies suggests an analysis threshold of 175RFU under their default injection conditions. However, individual laboratories should determine their peak amplitude thresholds from internal validation studies. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes.
3. The normalization box can be checked regardless of whether normalization was or was not applied during data collection.

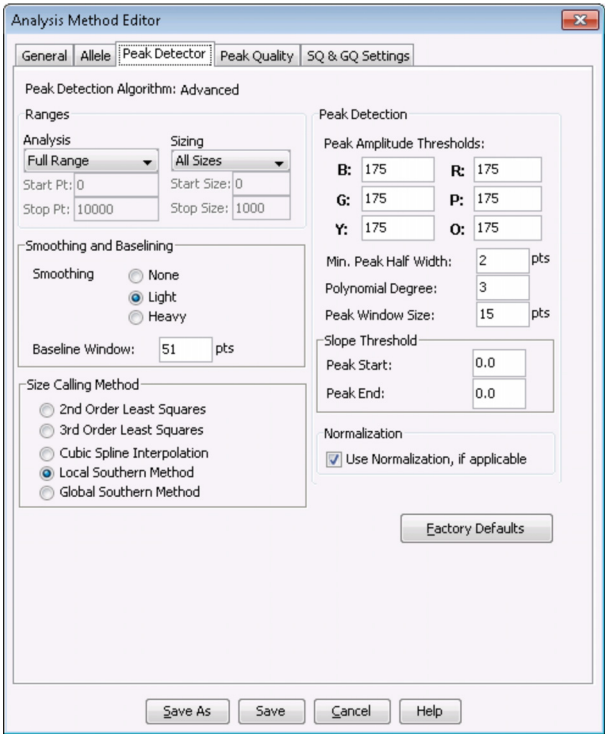

Figure 14. The GeneMapper® *ID*-X Peak Detector tab.

6.D. Creating a Casework Analysis Method with GeneMapper® *ID*-X Software, Version 1.2 (continued)

11. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 11 and 12, see the GeneMapper® *ID*-X user's manual for more information.

12. Select the SQ & GQ Settings tab. You may change these settings.

13. Select "Save" to save the new analysis method.

14. Select "Done" to exit the GeneMapper® *ID*-X Manager.

Processing Data for Casework Samples

1. Select "File", then "New Project".

2. Select "Edit", then "Add Samples to Project".

3. Browse to the location of the run files. Highlight desired files, then select "Add to list" followed by "Add".

4. In the Sample Type column, use the drop-down menu to select "Allelic Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Allelic Ladder" in the Sample Type column for proper genotyping.

   Note: The positive control DNA defined in the GeneMapper® *ID*-X panel file is the 2800M Control DNA. Redefine the genotype in the panel file if using a different positive control DNA.

5. In the Analysis Method column, select the analysis method created above.

6. In the Panel column, select the panels text file that was imported in Section 6.A.

7. In the Size Standard column, select the size standard that was imported in Section 6.B or created in Section 6.C.

8. Select "Analyze" (green arrow button) to start data analysis.

   Note: By default, the software displays the Analysis Requirement Summary, Allelic Ladder Analysis Summary and Analysis Summary windows after quality review by the software. Ensure that all requirements are met as each window appears. If you do not have the Analysis Requirement Summary window activated, you may need to do additional manual troubleshooting.

9. If all analysis requirements are met, the Save Project window will open (Figure 15).

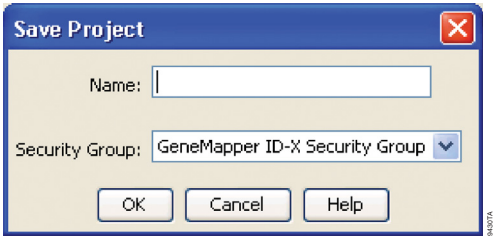

Figure 15. The Save Project window.

10. Enter the project name.
11. Choose the applicable security group from the drop-down menu, then select "OK".

Note: Sizing of Penta E and DYS391 alleles ≥475 bases will not use Local Southern Method. For Penta E, alleles >24 will be labeled as "OL."

When the analysis is finished, the Analysis Summary screen will appear. We recommend that you review any yellow or red marker header bars in the plots view and handle them according to laboratory standard operating procedures. Navigate to the Genotype tab or Samples tab. To assist the review of any low-quality samples, use the default Data Interpretation plot settings and review the contents in the Quality Value Details table.

The values displayed in the Analysis Method Peak Quality and SQ & GQ Settings tabs are defaults and will affect the quality values displayed in the plot settings. We recommend that you modify the values in these tabs to fit your laboratory's data analysis protocols.

6.E. Creating a Databasing or Paternity Analysis Method with GeneMapper® *ID*-X Software, Version 1.2

These instructions are intended as a guide to start analyzing data in GeneMapper® *ID*-X software. They are not intended as a comprehensive guide for using the GeneMapper® *ID*-X software. We recommend that users contact Applied Biosystems for training on the software.

1. Select "Tools", then "GeneMapper ID-X Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.
4. In the Analysis Method Editor window, select "GeneMapper ID-X Security Group" as the Security Group. This allows access to all users of the software. Other security groups may be used.
5. Enter a descriptive name for the analysis method, such as "PowerPlex Fusion 20% Filter".

6. Select the Allele tab (Figure 16).
7. Select the bins text file that was imported in Section 6.A.
8. We recommend the values shown in Figure 16 for proper filtering of stutter peaks when using the PowerPlex® Fusion System. You may need to optimize these settings. In-house validation should be performed.

   Note: Ensure that the appropriate 20% filter is applied to this analysis method by entering "0.20" for the Global Cut-off Value for Tri, Tetra and Penta repeats.

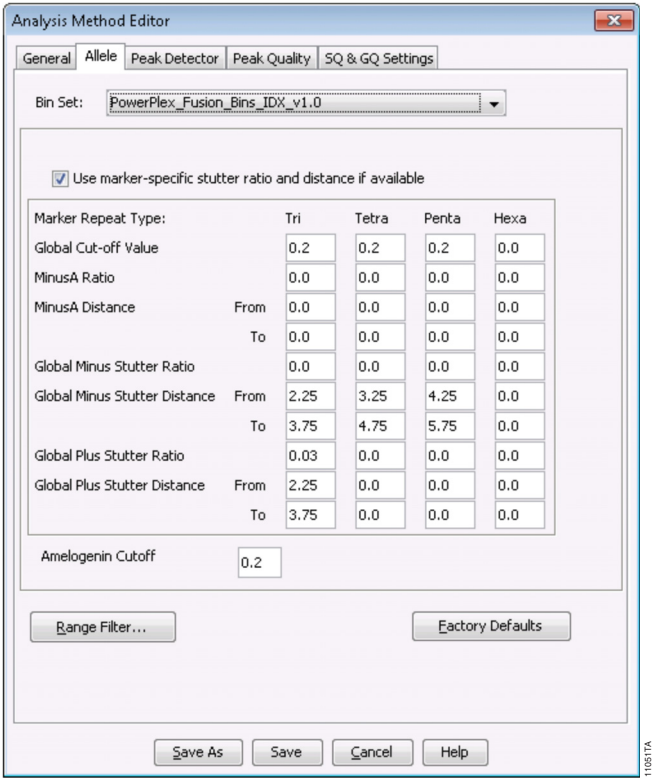

Figure 16. The GeneMapper® *ID*-X Allele tab.

9. Select the Peak Detector tab. Figure 14 shows an example of settings used at Promega. You may need to optimize these settings. In-house validation should be performed.

   Notes:

   1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.
   2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds are usually 50–150RFU on the ABI PRISM® 3100 and 3100-*Avan*t Genetic Analyzers and Applied Biosystems® 3130 and 3130*xl* Genetic Analyzers. For the Applied Biosystems® 3500 and 3500xL Genetic Analyzers, Life Technologies suggests an analysis threshold of 175RFU under their default injection conditions. However, individual laboratories should determine their peak amplitude thresholds from internal validation studies. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes.
   3. The normalization box can be checked regardless of whether normalization was or was not applied during data collection.

10. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 10 and 11, see the GeneMapper® *ID*-X user's manual for more information.

11. Select the SQ & GQ Settings tab. You may change these settings.

12. Select "Save" to save the new analysis method.

13. Select "Done" to exit the GeneMapper® *ID*-X Manager.

Processing Data for Databasing or Paternity Samples

1. Select "File", then "New Project".

2. Select "Edit", then "Add Samples to Project".

3. Browse to the location of run files. Highlight desired files, then select "Add to list" followed by "Add".

4. In the Sample Type column, use the drop-down menu to select "Allelic Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Allelic Ladder" in the Sample Type column for proper genotyping.

   Note: The positive control DNA defined in the GeneMapper® *ID*-X panel file is the 2800M Control DNA. Redefine the genotype in the panel file if using a different positive control DNA.

   In the Analysis Method column, select the analysis method created above.

6.E. Creating a Databasing or Paternity Analysis Method with GeneMapper® *ID-X* Software, Version 1.2 (continued)

5. In the Panel column, select the panels text file that was imported in Section 6.A.
6. In the Size Standard column, select the size standard that was imported in Section 6.B or created in Section 6.C.
7. Select "Analyze" (green arrow button) to start data analysis.

   Note: By default, the software displays the Analysis Requirement Summary, Allelic Ladder Analysis Summary and Analysis Summary windows after quality review by the software. Ensure that all requirements are met as each window appears. If you do not have the Analysis Requirement Summary window activated, you may need to do additional manual troubleshooting.

8. If all analysis requirements are met, the Save Project window will open (Figure 15).
9. Enter the project name.
10. Choose the applicable security group from the drop-down menu, then select "OK".

Note: Sizing of Penta E and DYS391 alleles ≥475 bases will not use Local Southern Method. For Penta E, alleles >24 will be labeled as "OL."

When the analysis is finished, the Analysis Summary screen will appear. We recommend that you review any yellow or red marker header bars in the plots view and handle them according to laboratory standard operating procedures. Navigate to the Genotype tab or Samples tab. To assist the review of any low-quality samples, use the default Data Interpretation plot settings and review the contents in the Quality Value Details table.

The values displayed in the Analysis Method Peak Quality and SQ & GQ Settings tabs are defaults and will affect the quality values displayed in the plot settings. We recommend that you modify the values in these tabs to fit your laboratory's data analysis protocols.

6.F. Importing PowerPlex® Fusion Panels and Bins Text Files with GeneMapper® *ID* Software, Version 3.2

To facilitate analysis of data generated with the PowerPlex® Fusion System, we have created panels and bins text files to allow automatic assignment of genotypes using GeneMapper® *ID* software, version 3.2. We recommend that users of GeneMapper® *ID* software, version 3.2, complete the *Applied Biosystems GeneMapper®* ID *Software Human Identification Analysis Tutorial* to familiarize themselves with proper operation of the software. For GeneMapper® *ID* software, version 3.1, users we recommend upgrading to version 3.2.

For analysis using GeneMapper® *ID* software, version 3.2, you will need the proper panels and bins text files: PowerPlex_Fusion_Panels_vX.x.txt and PowerPlex_Fusion_Bins_vX.x.txt files, where "X.x" refers to the most recent version of the panels and bins text files.

Getting Started

1. To obtain the proper panels and bins text files for the PowerPlex® Fusion System go to: **www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/**
2. Enter your contact information, and select "GeneMapper ID". Select "Submit".
3. Save the PowerPlex_Fusion_Panels_IDX_vX.x.txt and PowerPlex_Fusion_Bins_IDX_vX.x.txt files, where "X.x" refers to the most recent version of the panels and bins text files, to a known location on your computer.

Importing Panels and Bins Text Files

These instructions loosely follow the Applied Biosystems GeneMapper® *ID* software tutorial, pages 1–4.

1. Open the GeneMapper® *ID* software, version 3.2.
2. Select "Tools", then "Panel Manager".
3. Highlight the Panel Manager icon in the upper left navigation pane.
4. Select "File", then "Import Panels".
5. Navigate to the panels text file imported in the Getting Started section above. Select the file, then "Import".
6. In the navigation pane, highlight the PowerPlex Fusion panels folder that you just imported in Step 5.
7. Select "File", then "Import Bin Set".
8. Navigate to the bins text file imported in the Getting Started section above. Select the file, then "Import".
9. At the bottom of the Panel Manager window, select "OK". The Panel Manager window will close automatically.

6.G. Importing the CC5 ILS 500 Size Standard into GeneMapper® *ID* Software, Version 3.2

There are two options when creating a size standard. Use this protocol or the alternative protocol in Section 6.H.

The CC5_ILS_500.xml file is available for download at:
www.promega.com/resources/tools/genemapper-id-software-panels-and-bin-sets/

Save the CC5_ILS_500.xml file to a known location on your computer.

1. Select "Tools", then "GeneMapper Manager".
2. Select the Size Standard tab.
3. Select "Import".
4. Browse to the location of the CC5_ILS_500.xml file.
5. Highlight the file, then select "Import".
6. Select "Done" to save changes and exit the GeneMapper Manager.

6.H. Creating a Size Standard with GeneMapper® *ID* Software, Version 3.2

1. Select "Tools", then "GeneMapper Manager".
2. Select the Size Standard tab.
3. Select "New".
4. Select "Basic or Advanced" (Figure 17). The type of analysis method selected must match the type of analysis method created earlier. Select "OK".

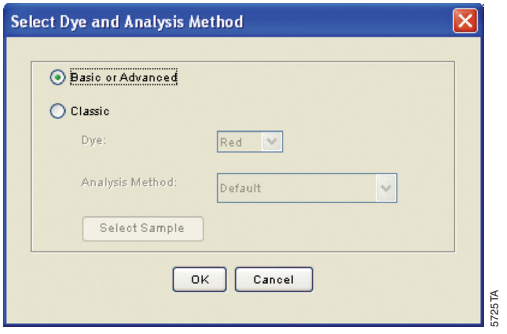

Figure 17. The Select Dye and Analysis Method window.

5. Enter a detailed name, such as "CC5 ILS 60 to 500", in the Size Standard Editor (Figure 18).
6. Choose "Orange" for the Size Standard Dye.
7. Enter the sizes of the internal lane standard fragments (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases). See Section 9.C, Figure 24.
8. Select "OK".

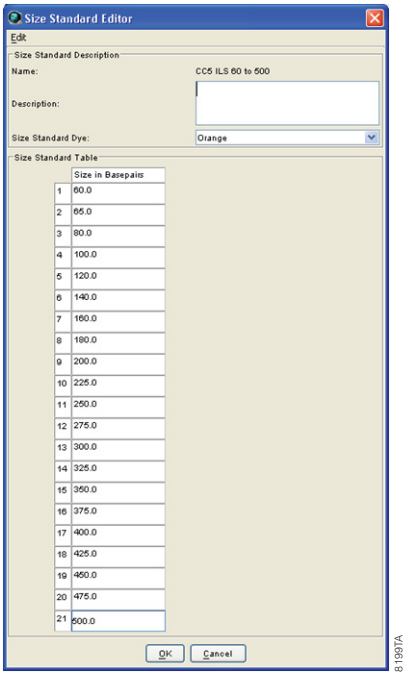

Figure 18. The Size Standard Editor.

6.I. Creating a Casework Analysis Method with GeneMapper® *ID* Software, Version 3.2

These instructions loosely follow the Applied Biosystems GeneMapper® *ID* software tutorial, pages 5–11.

1. Select "Tools", then "GeneMapper Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.
4. Select "HID", and select "OK".

   Note: If you do not see the HID option, you do not have the GeneMapper® *ID* software. Contact Applied Biosystems.

5. Enter a descriptive name for the analysis method, such as "PowerPlex Fusion".
6. Select the Allele tab (Figure 19).
7. Select the bins text file that was imported in Section 6.F.
8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.
9. Enter the values shown in Figure 19 for proper filtering of stutter peaks when using the PowerPlex® Fusion System. For an explanation of the proper usage and effects of these settings, refer to the Applied Biosystems user bulletin titled "*Installation Procedures and New Features for GeneMapper* ID *Software 3.2*".

   Note: Some of these settings have been optimized and are different from the recommended settings in the user bulletin.

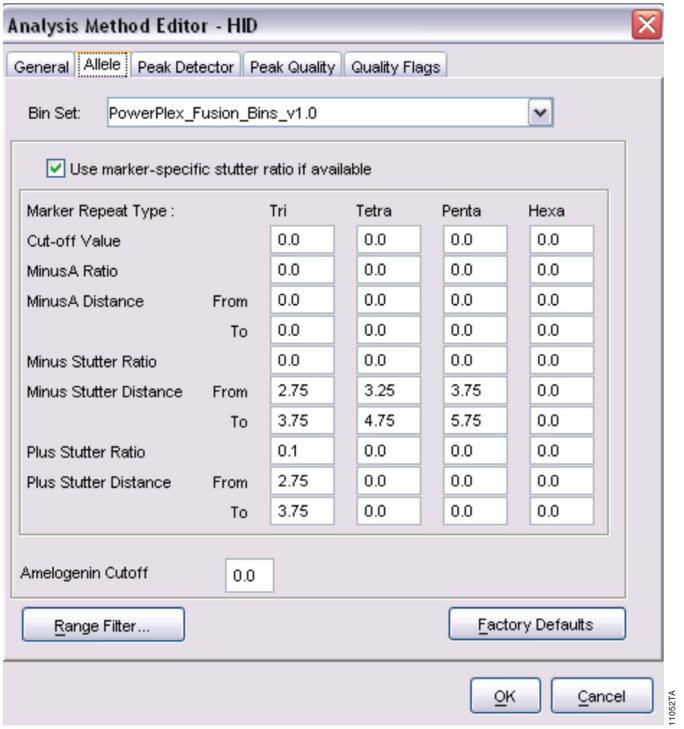

Figure 19. The GeneMapper® *ID* Allele tab.

10. Select the Peak Detector tab. We recommend the settings shown in Figure 20.

    Notes:

    1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.
    2. The peak amplitude thresholds are the minimum peak heights at which the software will call a peak. Values for peak amplitude thresholds are usually 50–150RFU and should be determined by individual laboratories. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes.

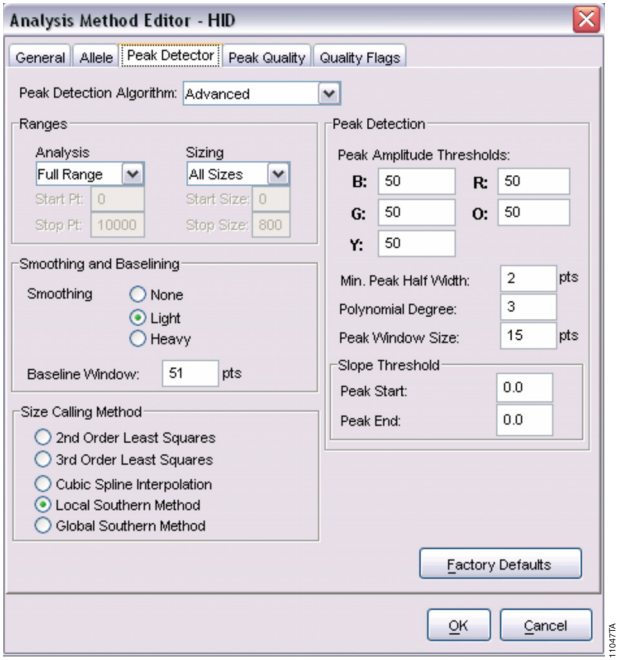

Figure 20. The GeneMapper® *ID* Peak Detector tab.

11. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 11 and 12, see the GeneMapper® *ID* user's manual for more information.

12. Select the Quality Flags tab. You may change these settings.

13. Select "OK" to save your settings.

6.I. Creating a Casework Analysis Method with GeneMapper® *ID* Software, Version 3.2 (continued)

Processing Data for Casework Samples

1. Select "File", then "New Project".
2. Select "Edit", then "Add Samples to Project".
3. Browse to the location of the run files. Highlight desired files, then select "Add to list" followed by "Add".
4. In the Sample Type column, use the drop-down menu to select "Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Ladder" in the Sample Type column for proper genotyping.

   Note: The positive control DNA defined in the GeneMapper® *ID* panel file is the 2800M Control DNA. Redefine the genotype in the panel file if using a different positive control DNA.
5. In the Analysis Method column, select the analysis method created previously in this section.
6. In the Panel column, select the panels text file that was imported in Section 6.F.
7. In the Size Standard column, select the size standard that was imported in Section 6.G or created in Section 6.H.
8. Select "Analyze" (green arrow button) to start data analysis.

Note: Sizing of Penta E and DYS391 alleles ≥475 bases will not use Local Southern Method. For Penta E, alleles >24 will be labeled as "OL."

6.J. Creating a Databasing or Paternity Analysis Method with GeneMapper® *ID* Software, Version 3.2

1. Select "Tools", then "GeneMapper Manager".
2. Select the Analysis Methods tab.
3. Select "New", and a new analysis method dialog box will open.
4. Select "HID", and select "OK".

   Note: If you do not see the HID option, you do not have the GeneMapper® *ID* software. Contact Applied Biosystems.
5. Enter a descriptive name for the analysis method, such as "PowerPlex_Fusion_20%filter".
6. Select the Allele tab (Figure 21).
7. Select the bins text file that was imported in Section 6.F.
8. Ensure that the "Use marker-specific stutter ratio if available" box is checked.

9. Enter the values shown in Figure 21 for proper filtering of peaks when using the PowerPlex® Fusion System. For an explanation of the proper usage and effect of these settings, refer to the Applied Biosystems user bulletin titled "*Installation Procedures and New Features for GeneMapper* ID *Software* 3.2".

   Note: Ensure that the appropriate 20% filter is applied to this analysis method by entering "0.20" for the Global Cut-off Value for Tri, Tetra and Penta repeats.

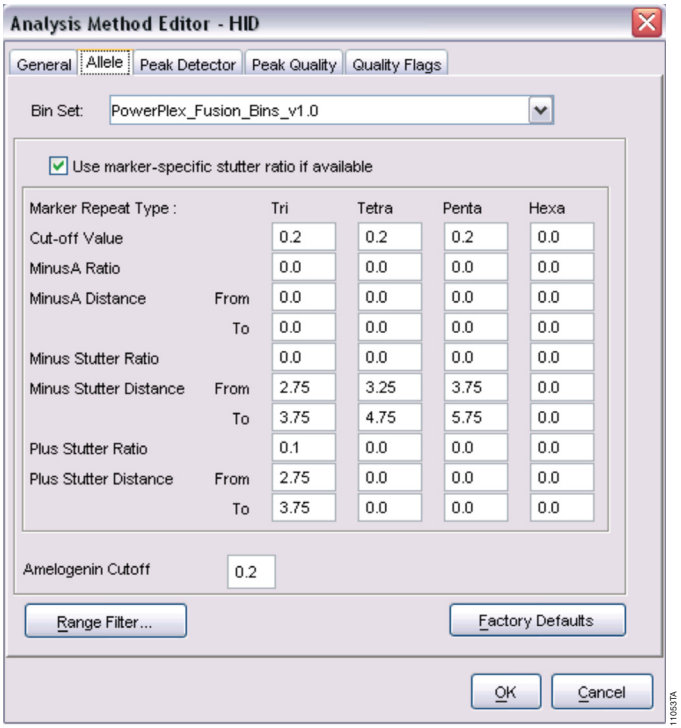

Figure 21. The GeneMapper® *ID* Allele tab with settings for using a 20% peak filter.

10. Select the Peak Detector tab. We recommend the settings shown in Figure 20.

    Notes:

    1. Select full range or partial range for the analysis range. When using a partial range, choose an appropriate analysis range based on your data. Choose a start point after the primer peak and just before the first defined internal lane standard peak to help ensure proper sizing of the internal lane standard.
    2. The peak amplitude thresholds are the minimum peak heights that the software will call as a peak. Values for peak amplitude thresholds are usually 50–150RFU and should be determined by individual laboratories. Peak heights for the CC5 ILS 500 are generally lower than those for the other dyes. Therefore, the threshold for the orange dye may be lower than that for the other dyes.

6.J. Creating a Databasing or Paternity Analysis Method with GeneMapper® *ID* Software, Version 3.2 (continued)

11. Select the Peak Quality tab. You may change the settings for peak quality.

    Note: For Steps 11 and 12, see the GeneMapper® *ID* user's manual for more information.

12. Select the Quality Flags tab. You may change these settings.

13. Select "OK" to save your settings.

Processing Data for Databasing or Paternity Samples

1. Select "File", then "New Project".

2. Select "Edit", then "Add Samples to Project".

3. Browse to the location of the run files. Highlight desired files, then select "Add to list" followed by "Add".

4. In the Sample Type column, use the drop-down menu to select "Ladder", "Sample", "Positive Control" or "Negative Control" as appropriate for the sample. Every folder in the project must contain at least one allelic ladder injection that is designated as "Ladder" in the Sample Type column for proper genotyping.

   Note: The positive control DNA defined in the GeneMapper® *ID* panel file is the 2800M Control DNA. Redefine the genotype in the panel file if using a different positive control DNA.

5. In the Analysis Method column, select the analysis method created previously in this section.

6. In the Panel column, select the panels text file that was imported in Section 6.F.

7. In the Size Standard column, select the size standard that was imported in Section 6.G or created in Section 6.H.

8. Select "Analyze" (green arrow button) to start the data analysis.

Note: Sizing of Penta E and DYS391 alleles ≥475 bases will not use Local Southern Method. For Penta E, alleles >24 will be labeled as "OL."

6.K. Controls

1. Observe the results for the negative control. Using the protocols defined in this manual, the negative control should be devoid of amplification products.

2. Observe the results for the 2800M Control DNA. Compare the 2800M DNA allelic repeat sizes with the locus-specific allelic ladder. The expected 2800M DNA allele designations for each locus are listed in Table 6 (Section 9.A).

6.L. Results

Representative results of the PowerPlex® Fusion System are shown in Figure 22. The PowerPlex® Fusion Allelic Ladder Mix is shown in Figure 23.

Artifacts and Stutter

Stutter products are a common amplification artifact associated with STR analysis. Stutter products often are observed one repeat unit below the true allele peak and, occasionally, two repeat units smaller or one repeat unit larger than the true allele peak. Frequently, alleles with a greater number of repeat units will exhibit a higher percent stutter. A trinucleotide repeat locus, like D22S1045, will have more pronounced stutter in both n–3 and n+3 positions than a typical tetranucleotide repeat locus. The pattern and intensity of stutter may differ slightly between primer sets for the same loci.

The mean plus three standard deviations at each locus is used in the PowerPlex® Fusion panels text file for locus-specific filtering in the GeneMapper® *ID* software, version 3.2, and in the PowerPlex® Fusion stutter text file for locus-specific filtering in GeneMapper® *ID*-X software.

In addition to stutter peaks, other artifact peaks can be observed at some of the PowerPlex® Fusion System loci. Low-level products can be seen in the n–2 and n+2 positions with some loci such as D1S1656, D13S317, D18S51, D21S11, D7S820, D5S818, D12S391 and D19S433. N–1 peaks are sometimes present at amelogenin and D2S441. N-3 peaks are sometimes present at D12S391. Amplification-independent artifacts may be observed in template and no-template samples in the fluorescein channel at 64–65, 69–71 and 88–90 bases and in the JOE channel at 74–76 bases. Artifact peaks may be seen outside the locus panels in the fluorescein channel at 70–74 bases, in the TMR-ET channel at 66–68 bases and in the CXR-ET channel at 58–65 bases. Artifacts that may be seen within the locus panels include allele 5 (84 bases) in D16S539 and peaks at 71–73 and 75–77 bases in TH01, 214 bases in D18S51 and 247 bases in D2S1338. These artifacts are typically below common minimum thresholds.

The PowerPlex® Fusion System is optimized for use with POP-4® polymer. This system was not developed for use with POP-7 polymer; if using POP-7™ polymer, optimization and in-house validation are required. Some DNA-independent artifacts specific to the PowerPlex® Fusion System with POP-7™ polymer have been noted. The global filters used for database analyses will generally filter these artifact peaks. Artifact peaks may be seen in the fluorescein channel at 74–76 bases, 85–87 bases and 99–101 bases. In the JOE channel, artifacts may be seen at 88–90 bases. The signal strength of the JOE-channel artifact increases with storage of the amplification plate at 4°C, most commonly when plates are left at 4°C for a few days. We recommend storing amplification products at –20°C.

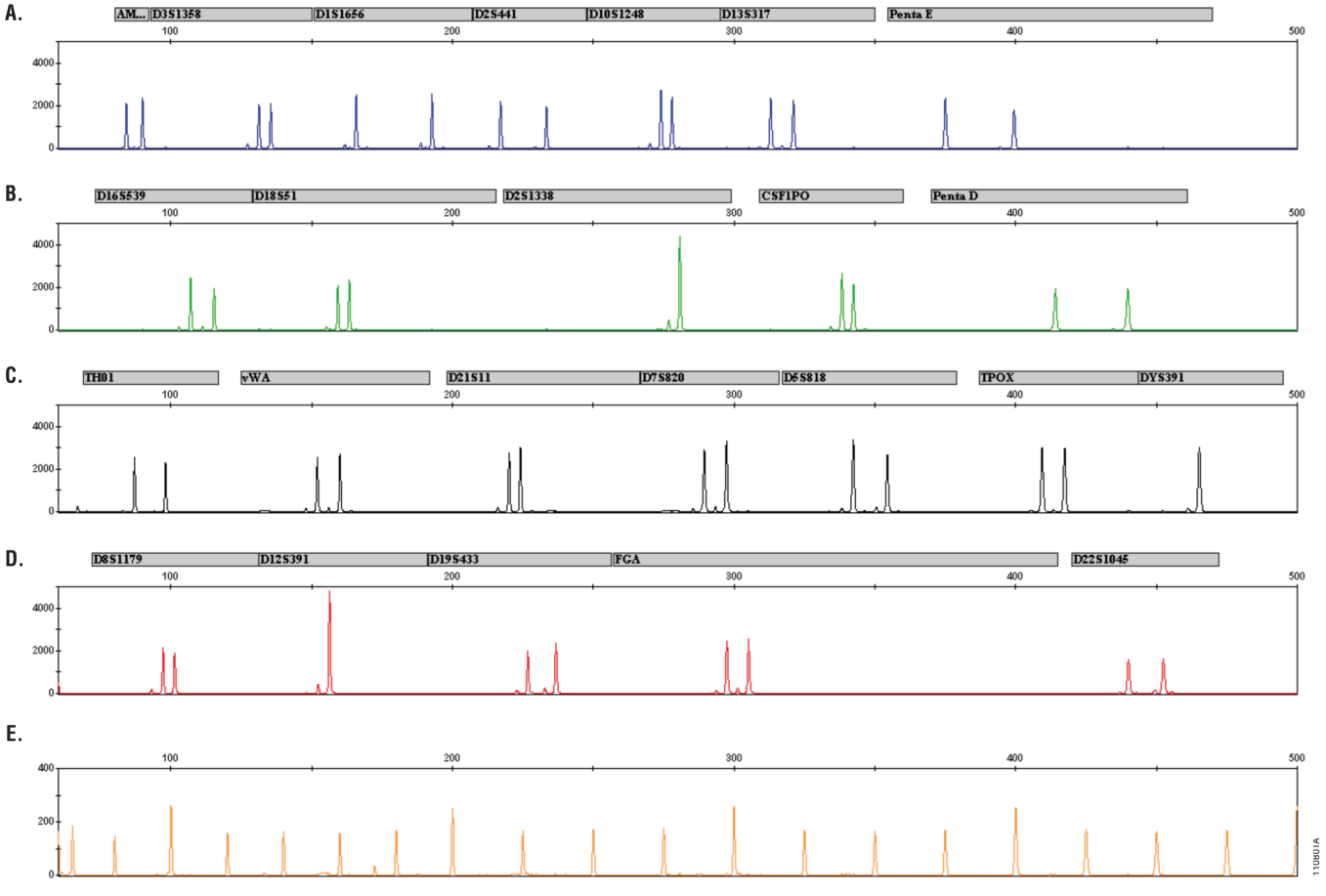

Figure 22. The PowerPlex® Fusion System. A single-source male template DNA (0.5ng) was amplified using the PowerPlex® Fusion System and 30 cycles. Amplification products were mixed with CC5 Internal Lane Standard 500 and analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 5-second injection. Results were analyzed using GeneMapper® *ID* software, version 3.2. **Panel A.** An electropherogram showing the peaks of the fluorescein-labeled loci: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317 and Penta E. **Panel B.** An electropherogram showing the peaks of the JOE-labeled loci: D16S539, D18S51, D2S1338, CSF1PO and Penta D. **Panel C.** An electropherogram showing the peaks of the TMR-ET-labeled loci: TH01, vWA, D21S11, D7S820, D5S818, TPOX and DYS391. **Panel D.** An electropherogram showing the peaks of the CXR-ET-labeled loci: D8S1179, D12S391, D19S433, FGA and D22S1045. **Panel E.** An electropherogram showing the 60bp to 500bp fragments of the CC5 Internal Lane Standard 500.

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Figure 23. The PowerPlex® Fusion Allelic Ladder Mix. The PowerPlex® Fusion Allelic Ladder Mix was analyzed with an Applied Biosystems® 3130 Genetic Analyzer using a 3kV, 3-second injection. The sample file was analyzed with the GeneMapper® *ID* software, version 3.2, and PowerPlex® Fusion panels and bins text files. **Panel A.** The fluorescein-labeled allelic ladder components and their allele designations. **Panel B.** The JOE-labeled allelic ladder components and their allele designations. **Panel C.** The TMR-ET-labeled allelic ladder components and their allele designations. **Panel D.** The CXR-ET-labeled allelic ladder components and their allele designations.

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **genetic@promega.com**

7.A. Amplification and Fragment Detection

This section provides information about general amplification and detection. For questions about amplification of extracted DNA, see Section 7.B. For questions about direct amplification, see Sections 7.C and 7.D.

Symptoms	Causes and Comments
Faint or absent allele peaks	The PowerPlex® Fusion 5X Master Mix was not vortexed well before use. Vortex the 5X Master Mix for 15 seconds before dispensing into the PCR amplification mix.
	An air bubble formed at the bottom of the reaction tube. Use a pipette to remove the air bubble, or centrifuge the reactions briefly before thermal cycling.
	Thermal cycler, plate or tube problems. Review the thermal cycling protocol in Section 4. We have not tested other reaction tubes, plates or thermal cyclers. Calibrate the thermal cycler heating block if necessary.
	Primer concentration was too low. Use the recommended primer concentration. Vortex the PowerPlex® Fusion 5X Primer Pair Mix for 15 seconds before use.
	Poor capillary electrophoresis injection (CC5 ILS 500 peaks also affected). Re-inject the sample. Check the syringe pump system for leakage. Check the laser power.
	Samples were not denatured completely. Heat-denature samples for the recommended time, then cool on crushed ice or in an ice-water bath immediately prior to capillary electrophoresis. Do not cool samples in a thermal cycler set at 4°C, as this may lead to artifacts due to DNA re-annealing.
	Poor-quality formamide was used. Use only Hi-Di™ formamide when analyzing samples.
Extra peaks visible in one or all color channels	Contamination with another template DNA or previously amplified DNA. Cross-contamination can be a problem. Use aerosol-resistant pipette tips, and change gloves regularly.
	Samples were not denatured completely. Heat denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to loading the capillary.
	Double-stranded DNA migrates faster than single-stranded DNA during capillary electrophoresis. Appearance of "shadow" peaks migrating in front of the main peaks, especially if the shadow peaks are separated by the same distance as the main peaks in a heterozygote, can indicate the presence of double-stranded DNA due to incomplete denaturation or post-injection re-annealing.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. Be sure to perform an extension step (10 minutes for purified DNA samples) at 60°C after thermal cycling (Section 4).

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels (continued)	CE-related artifacts ("spikes"). Minor voltage changes or urea crystals passing by the laser can cause "spikes" or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject samples to confirm.
	Incorrect G5 spectral was active. Re-run samples, and confirm that the PowerPlex® 5-dye G5 spectral is set for G5. See instructions on instrument preparation in Section 5.
	Pull-up or bleedthrough. Pull-up can occur when peak heights are too high or if a poor or incorrect matrix is applied to the samples. • Perform a new spectral calibration, and re-run the samples. • Instrument sensitivities can vary. Optimize the injection conditions. See Section 5.
	CE-related artifacts (contaminants). Contaminants in the water used with the instrument or to dilute the 10X genetic analyzer buffer may generate peaks in the fluorescein and JOE channels. Use autoclaved deionized water; change vials and wash buffer reservoir.
	Repeat sample preparation using fresh formamide. Long-term storage of amplified sample in formamide can result in artifacts.
	The CE polymer was beyond its expiration date, or polymer was stored at room temperature for more than one week.
	Maintain instrumentation on a daily or weekly basis, as recommended by the manufacturer.
	POP-7™-related artifacts. This system was not developed for use with POP-7 polymer; if using POP-7™ polymer, optimization and in-house validation are required. The use of POP-7™ CE polymer can change the migration and sizing location of artifacts compared to the POP-4® locations. An artifact can be seen with the POP-7™ polymer at 88–90bp in the JOE channel and at 74–76, 85–87 or 99–101bp in the fluorescein channel.
Allelic ladder not running the same as samples	Allelic ladder and primer pair mix were not compatible. Ensure that the allelic ladder is from the same kit as the primer pair mix.
	Poor-quality formamide. Use only Hi-Di™ formamide when analyzing samples.
	Be sure the allelic ladder and samples are from the same instrument run.
	Migration of samples changed slightly over the course of a CE run with many samples. This may be due to changes in temperature or the CE column over time. Use a different injection of allelic ladder to determine sizes.
	Poor injection of allelic ladder. Include more than one ladder per instrument run.
	Internal size standard not assigned correctly. Evaluate the sizing labels on the CC5 ILS 500 and correct if necessary.

7.A. Amplification and Fragment Detection (continued)

Symptoms	Causes and Comments
Peak height imbalance	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in DNA samples. Decreasing the reaction volume can result in suboptimal performance.
	Miscellaneous balance problems. Thaw the 5X Primer Pair Mix and 5X Master Mix completely, and vortex for 15 seconds before use. Note that the 5X Master Mix will take longer to thaw than the 5X Primer Pair Mix. Do not centrifuge the 5X Primer Pair Mix or 5X Master Mix after mixing. Calibrate thermal cyclers and pipettes routinely.
	PCR amplification mix prepared in Section 4 was not mixed well. Vortex the PCR amplification mix for 5–10 seconds before dispensing into the reaction tubes or plate.

7.B. Amplification of Extracted DNA

The following information is specific to amplification of extracted DNA. For information about general amplification and detection, see Section 7.A.

Symptoms	Causes and Comments
Faint or absent allele peaks	Impure template DNA. Because a small amount of template is used, this is rarely a problem. Depending on the DNA extraction procedure used and sample source, inhibitors might be present in the DNA sample.
	Insufficient template. Use the recommended amount of template DNA if available.
	High salt concentration or altered pH. If the DNA template is stored in TE buffer that is not pH 8.0 or contains a higher EDTA concentration, the DNA volume should not exceed 20% of the total reaction volume. Carryover of K^+, Na^+, Mg^{2+} or EDTA from the DNA sample can negatively affect PCR. A change in pH also may affect PCR. Store DNA in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) or TE^{-4} buffer with 20µg/ml glycogen.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl. Decreasing the reaction volume may result in suboptimal performance.
Extra peaks visible in one or all color channels	Artifacts of STR amplification. Amplification of excess amounts of purified DNA can result in a higher number of artifact peaks. Use the recommended amount of template DNA. See Section 6.L for additional information on stutter and artifacts.
Peak height imbalance	Excessive amount of DNA. Amplification of >0.5ng of template can result in an imbalance, with smaller loci showing more product than larger loci. Decrease number of cycles.
	Degraded DNA sample. DNA template was degraded, and larger loci showed diminished yield.
	Insufficient template DNA. Use the recommended amount of template DNA if available. Stochastic effects can occur when amplifying low amounts of template.
	Impure template DNA. Inhibitors that may be present in forensic samples can lead to allele dropout or imbalance.

7.C. Direct Amplification of DNA From Storage Card Punches

The following information is specific to direct amplification of DNA from storage card punches. For additional information about general amplification and detection, see Section 7.A.

Symptoms	Causes and Comments
Faint or absent allele peaks	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in FTA® cards and DNA samples. Decreasing the reaction volume may result in suboptimal performance.
	Poor sample transfer to storage card or variable sampling from storage card. Take punches from a different portion of the card. Increasing cycle number can improve low peak heights.
	DNA was not accessible on nonlytic material. Pretreat nonFTA materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
	Too much sample in the reaction. Use one or two 1.2mm storage card punches. Follow the manufacturer's recommendations when depositing sample onto the storage card. With FTA® cards, reducing the reaction volumes below 25µl may result in amplification failure.
	Positive control did not amplify. Do not include a blank punch in the positive control reaction. Presence of blank punches may inhibit amplification of 2800M Control DNA.
Extra peaks visible in one or all color channels	Punch may be contaminated. Take punches from blank paper between samples.
	Artifacts of STR amplification. Direct amplification of >20ng of template can result in a higher number of artifact peaks. Use the recommended punch size and number. See Section 6.L for additional information on stutter and artifacts.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. • Be sure to perform a 20-minute extension step at 60°C after thermal cycling (Section 4.B). • Decrease cycle number. • Increase the final extension time.
Peak height imbalance	Excessive amount of DNA. Amplification of >20ng of template can result in an imbalance, with smaller loci showing more product than larger loci. • Use one or two 1.2mm punches from a storage card containing a buccal sample or one 1.2mm punch from a storage card containing whole blood. Follow the manufacturer's recommendations when depositing sample onto the card. • Decrease cycle number.
	The reaction volume was too low. This system is optimized for a final reaction volume of 25µl to overcome inhibitors present in FTA® cards and PunchSolution™ Reagent. Decreasing the reaction volume can result in suboptimal performance.
	Amplification was inhibited when using more than one storage card punch with blood. Use only one 1.2mm storage card punch with blood.

7.C. Direct Amplification of DNA From Storage Card Punches (continued)

Symptoms	Causes and Comments
Peak height imbalance (continued)	DNA was not accessible on nonlytic material. Small loci may amplify preferentially, with large loci dropping out. Pretreat nonFTA materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.

7.D. Direct Amplification of DNA From Swabs

The following information is specific to direct amplification of DNA from swabs after pretreatment using the SwabSolution™ Kit. For additional information about general amplification and detection, see Section 7.A.

Symptoms	Causes and Comments
Faint or absent allele peaks	Poor sample deposition. Shedding and collection of donor cells was variable. Increase cycle number.
	Inactive SwabSolution™ Reagent. Thaw the SwabSolution™ Reagent completely in a 37°C water bath, and mix by gentle inversion. Store the SwabSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not refreeze; avoid multiple freeze-thaw cycles, as this may reduce activity.
	Active SwabSolution™ Reagent carried over into the amplification reaction. Ensure that the heat block is heating to 70°C (90°C if using a 2.2ml, Square-Well Deep Well Plate) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator set at 70°C to incubate tubes or plates; heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer. We have tested 60-minute incubation times and observed no difference in performance compared to a 30-minute incubation.
	DNA was not accessible on nonlytic material. Pretreat nonFTA materials with SwabSolution™ Reagent to ensure that DNA is liberated from cellular proteins.
Faint or absent peaks for the positive control reaction	If the positive control reaction failed to amplify, check to make sure that the correct amount of 2800M Control DNA was added to the reaction. Due to the reduced cycle numbers used with swab extracts, it is necessary to increase the mass of 2800M Control DNA to obtain a profile. We recommend 10ng of 2800M Control DNA per 25µl amplification reaction. This mass of DNA should be reduced if the cycle number used is increased and decreased if the cycle number is increased. Increase or decrease by twofold the mass of 2800M Control DNA for every one-cycle decrease or increase, respectively.
	Improper storage of the 2800M Control DNA.

Symptoms	Causes and Comments
Extra peaks visible in one or all color channels	Swab extract was contaminated. Assemble a reaction containing the swab extract prepared from a blank swab, or assemble a reaction where the SwabSolution™ Reagent is processed and incubated as a blank without a swab.
	Artifacts of STR amplification. Amplification of swab extracts with high DNA concentrations can result in artifact peaks due to overamplification, resulting in saturated signal on the CE instrument. We recommend 2µl of swab extract per 25µl reaction. Using more than 2µl in a 25µl reaction or using 2µl with a smaller reaction volume may result in overamplification and signal saturation. If signal is saturated, repeat amplification with less swab extract or reduced cycle number.
	Artifacts of STR amplification. Amplification of STRs can result in artifacts that appear as peaks one base smaller than the allele due to incomplete addition of the 3´ A residue. • Be sure to perform the 20-minute extension step at 60°C after thermal cycling (Section 4.C) • Use 2µl of swab extract in a 25µl PowerPlex® Fusion reaction. A larger volume of swab extract may contain more than the recommended amount of DNA template, resulting in incomplete adenylation. • Decrease cycle number. • Increase the final extension time.
Peak height imbalance	Excess DNA in the amplification reaction can result in locus-to-locus imbalance within a dye channel such that the peak heights at the smaller loci are greater than those at the larger loci (ski-slope effect). Use less swab extract, or reduce cycle number.
	Active SwabSolution™ Reagent carried over from swab extracts into the amplification reaction. Larger loci are most susceptible to reagent carryover and will drop out before the smaller loci. Ensure that the heat block is heating to 70°C (90°C if using 2.2ml, Square-Well Deep Well Plates) and samples were incubated for the full 30 minutes. Incubation for shorter time periods may result in incomplete reagent inactivation. Do not use an incubator set at 70°C to incubate tubes or plates; heat transfer is inefficient and will result in poor performance. Use only a heat block to maintain efficient heat transfer.
	Inactive SwabSolution™ Reagent. Thaw the SwabSolution™ Reagent completely in a 37°C water bath, and mix by gentle inversion. Store the SwabSolution™ Reagent at 2–10°C. Do not store reagents in the refrigerator door, where the temperature can fluctuate. Do not re-freeze; avoid multiple freeze-thaw cycles, as this may reduce activity.
	DNA was not accessible on nonlytic material. Small loci may amplify preferentially, with large loci dropping out. Pretreat nonFTA materials with PunchSolution™ Reagent to ensure that DNA is liberated from cellular proteins.

7.E. GeneMapper® *ID*-X Software

Symptoms	Causes and Comments
Stutter peaks not filtered	Stutter text file was not imported into the Panel Manager when the panels and bins text files were imported.
	Be sure that the "Use marker-specific stutter ratio and distance if available" box is checked.
	Stutter distance was not defined in the Analysis Method Allele tab.
Samples in the project not analyzed	The Analysis Requirement Summary window was not active, and there was an analysis requirement that was not met. Turn on Analysis Requirement Summary in the Options menu, and correct the necessary analysis requirements to continue analysis.
Edits in label edit viewer cannot be viewed	To view edits made to a project, the project first must be saved. Close the plot view window, return to the main GeneMapper® *ID*-X page and save the project. Display the plot window again, then view the label edit table.
Marker header bar for some loci are gray	When an edit is made to a locus, the quality flags and marker header bar automatically change to gray. To change the GQ and marker header bar for a locus to green, override the GQ in the plot window.
Alleles not called	To analyze samples with GeneMapper® *ID*-X software, at least one allelic ladder must be defined.
	An insufficient number of CC5 ILS 500 fragments was defined. Be sure to define at least two CC5 ILS 500 fragments smaller than the smallest sample peak and at least two CC5 ILS 500 fragments larger than the largest sample peak. In this instance, the allelic ladder would have failed the allelic ladder quality check.
	Run was too short, and larger peaks in ILS were not captured. Not all CC5 ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® *ID*-X software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze, as described in Section 6.D or 6.E.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the Sample Type column.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.

Symptoms	Causes and Comments
Off-ladder alleles (continued)	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
Size standard not called correctly	Starting data point was incorrect for the partial range chosen in Section 6.E. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.
	Extra peaks in size standard. Open the Size Match Editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes".
	Run was too short, and larger peaks in ILS were not captured. Not all CC5 ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
Peaks in size standard missing	If peaks are below threshold, decrease the peak amplitude threshold in the analysis method for the orange channel to include peaks.
	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration, and re-run the samples.
	Incorrect G5 spectral was active. Re-run samples, and confirm that the PowerPlex® 5-dye G5 spectral is set for G5. See instructions for instrument preparation in Section 5.

7.F. GeneMapper® *ID* Software

Symptoms	Causes and Comments
Alleles not called	To analyze samples with GeneMapper® *ID* software, the analysis parameters and size standard must both have "Basic or Advanced" as the analysis type. If they are different, an error is obtained.
	To analyze samples with GeneMapper® *ID* software, at least one allelic ladder must be defined.
	An insufficient number of CC5 ILS 500 fragments was defined. Be sure to define at least two CC5 ILS 500 fragments smaller than the smallest sample peak and at least two CC5 ILS 500 fragments larger than the largest sample peak.
	Run was too short, and larger peaks in ILS were not captured. Not all CC5 ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.

Symptoms	Causes and Comments
Off-ladder alleles	An allelic ladder from a different run than the samples was used. Re-analyze samples with an allelic ladder from the same run.
	The GeneMapper® *ID* software requires that the allelic ladder be imported from the same folder as the sample. Be sure that the allelic ladder is in the same folder as the sample. Create a new project and re-analyze as described in Section 6.I or 6.J.
	Panels text file selected for analysis was incorrect for the STR system used. Assign correct panels text file that corresponds to the STR system used for amplification.
	The allelic ladder was not identified as an allelic ladder in the Sample Type column.
	The wrong analysis type was chosen for the analysis method. Be sure to use the HID analysis type.
	The internal lane standard was not properly identified in the sample. Manually redefine the sizes of the size standard fragments in the sample.
	A low-quality allelic ladder was used during analysis. Ensure that only high-quality allelic ladders are used for analysis.
Size standard not called correctly	Starting data point was incorrect for the partial range chosen in Section 6.I. Adjust the starting data point in the analysis method. Alternatively, use a full range for the analysis.
	Extra peaks in advanced mode size standard. Open the Size Match Editor. Highlight the extra peak, select "Edit" and select "delete size label". Select "auto adjust sizes".
	Run was too short, and larger peaks in ILS were not captured. Not all CC5 ILS 500 peaks defined in the size standard were detected during the run. • Create a new size standard using the internal lane standard fragments present in the sample. • Re-run samples using a longer run time.
Peaks in size standard missing	If peaks are below threshold, decrease the peak amplitude threshold in the analysis method for the orange channel to include peaks.
	If peaks are low-quality, redefine the size standard for the sample to skip these peaks.
Error message: "Either panel, size standard, or analysis method is invalid"	The size standard and analysis method were not in the same mode ("Classic" vs. "Basic or Advanced"). Be sure both files are set to the same mode, either Classic or Basic or Advanced mode.
No alleles called, but no error message appears	Panels text file was not selected for sample. In the Panel column, select the appropriate panels text file for the STR system that was used.
	No size standard was selected. In the Size Standard column, be sure to select the appropriate size standard.
	Size standard was not correctly defined, or size peaks were missing. Redefine size standard to include only peaks present in your sample. Terminating analysis early or using short run times will cause larger ladder peaks to be missing. This will cause your sizing quality to be flagged as "red", and no allele sizes will be called.

Symptoms	Causes and Comments
Error message: "Both the Bin Set used in the Analysis Method and the Panel must belong to the same Chemistry Kit"	The bins text file assigned to the analysis method was deleted. In the GeneMapper Manager, select the Analysis Methods tab, and open the analysis method of interest. Select the Allele tab, and select an appropriate bins text file.
	The wrong bins text file was chosen in the analysis method Allele tab. Be sure to choose the appropriate bins text file, as shown in Figure 19.
Significantly raised baseline	Poor spectral calibration. Perform a new spectral calibration, and re-run the samples.
	Use of Classic mode analysis method. Use of Classic mode analysis on samples can result in baselines with more noise than those analyzed using the Basic or Advanced mode analysis method. Advanced mode analysis methods and size standards are recommended.
	Incorrect G5 spectral was active. Re-run samples, and confirm that the PowerPlex® 5-dye G5 spectral is set for G5. See instructions for instrument preparation in Section 5.
Error message after attempting to import panels and bins text files: "Unable to save panel data: java.SQLEException: ORA-00001: unique constraint (IFA.CKP_NNN) violated".	There was a conflict between different sets of panels and bins text files. Check to be sure that the bins are installed properly. If not, delete all panels and bins text files, and re-import files in a different order.
Allelic ladder peaks labeled off-ladder	GeneMapper® *ID* software was not used, or microsatellite analysis settings were used instead of HID analysis settings. GeneMapper® software does not use the same algorithms as GeneMapper® *ID* software and cannot correct for sizing differences using the allelic ladder. Promega recommends using GeneMapper® *ID* software to analyze PowerPlex® reactions. If using GeneMapper® *ID* software, version 3.2, be sure that the analysis method selected is an HID method. This can be verified by opening the analysis method using the GeneMapper Manager, then selecting the General tab. The analysis type cannot be changed. If the method is not HID, it should be deleted and a new analysis method created.

8. References

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9. Appendix

9.A. Advantages of Using the Loci in the PowerPlex® Fusion System

A single PowerPlex® Fusion System reaction amplifies all core loci required for US CODIS and European databases (Tables 4 and 5). Table 6 lists the PowerPlex® Fusion System alleles revealed in commonly available standard DNA templates. Additionally, the male-specific DYS391 locus is included to identify null Y results for Amelogenin.

We have carefully selected primers to avoid or minimize artifacts, including those associated with DNA polymerases, such as repeat slippage and terminal nucleotide addition (14,15). Repeat slippage, sometimes called "n–4 bands", "stutter" or "shadow bands", is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA, or both. The amount of this artifact observed depends primarily on the locus and the DNA sequence being amplified.

Terminal nucleotide addition (16,17) occurs when a thermostable nonproofeading DNA polymerase adds a nucleotide, generally adenine, to the 3´ ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact peak one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step at 60°C (18) to the amplification protocols to provide conditions for essentially complete terminal nucleotide addition when recommended amounts of template DNA are used.

Table 4. The PowerPlex® Fusion System Locus-Specific Information.

STR Locus	Label	Chromosomal Location[1]	Repeat Sequence[2] 5´ fi 3´
Amelogenin[3]	Fluorescein	Xp22.1–22.3 and Y	NA
D3S1358	Fluorescein	3p21.31 (45.557Mb)	TCTA Complex
D1S1656	Fluorescein	1q42 (228.972Mb)	TAGA Complex
D2S441	Fluorescein	2p14 (68.214Mb)	TCTA
D10S1248	Fluorescein	10q26.3 (130.567Mb)	GGAA
D13S317	Fluorescein	13q31.1 (81.62Mb)	TATC
Penta E	Fluorescein	15q26.2 (95.175Mb)	AAAGA
D16S539	JOE	16q24.1 (84.944Mb)	GATA
D18S51	JOE	18q21.33 (59.1Mb)	AGAA (19)
D2S1338	JOE	2q35 (218.705Mb)	TGCC/TTCC
CSF1PO	JOE	5q33.1 (149.436Mb)	AGAT
Penta D	JOE	21q22.3 (43.88Mb)	AAAGA
TH01	TMR-ET	11p15.5 (2.149Mb)	AATG (19)
vWA	TMR-ET	12p13.31 (5.963Mb)	TCTA Complex (19)
D21S11	TMR-ET	21q21.1 (19.476Mb)	TCTA Complex (19)
D7S820	TMR-ET	7q21.11 (83.433Mb)	GATA
D5S818	TMR-ET	5q23.2 (123.139Mb)	AGAT
TPOX	TMR-ET	2p25.3 (1.472Mb)	AATG
DYS391	TMR-ET	Y	TCTA
D8S1179	CXR-ET	8q24.13 (125.976Mb)	TCTA Complex (19)
D12S391	CXR-ET	12p12 (12.341Mb)	AGAT/AGAC Complex
D19S433	CXR-ET	19q12 (35.109Mb)	AAGG Complex
FGA	CXR-ET	4q28 (155.866Mb)	TTTC Complex (19)
D22S1045	CXR-ET	22q12.3 (35.779Mb)	ATT

[1]Information about the chromosomal location of these loci can be found in references 20, 21 and 22 and at: **www.cstl.nist.gov/biotech/strbase/chrom.htm**

[2]The August 1997 report (23,24) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, "1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5´ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used".

[3]Amelogenin is not an STR but displays an 89-base, X-specific band and a 95-base, Y-specific band.

Table 5. The PowerPlex® Fusion System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components[1,2] (bases)	Repeat Numbers of Allelic Ladder Components[3]
Amelogenin[4]	Fluorescein	89, 95	X, Y
D3S1358	Fluorescein	103–147	9–20
D1S1656	Fluorescein	161–208	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3
D2S441	Fluorescein	214–250	8–11, 11.3, 12–17
D10S1248	Fluorescein	256–280	8–19
D13S317	Fluorescein	302–350	5–17
Penta E	Fluorescein	371–466	5–24
D16S539	JOE	84–132	4–16
D18S51	JOE	134–214	7–10, 10.2, 11–13, 13.2, 14–27
D2S1338	JOE	224–296	10, 12, 14–28
CSF1PO	JOE	318–362	5–16
Penta D	JOE	377–450	2.2, 3.2, 5–17
TH01	TMR-ET	72–115	3–9, 9.3, 10–11, 13.3
vWA	TMR-ET	127–183	10–24
D21S11	TMR-ET	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38
D7S820	TMR-ET	269–313	5–16
D5S818	TMR-ET	321–369	6–18
TPOX	TMR-ET	393–441	4–16
DYS391	TMR-ET	442–486	5–16
D8S1179	CXR-ET	76–124	7–19
D12S391	CXR-ET	133–185	14–17, 17.3, 18, 18.3, 19–27
D19S433	CXR-ET	193–245	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2
FGA	CXR-ET	265–411	14–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2
D22S1045	CXR-ET	425–464	7–20

[1]The length of each allele in the allelic ladder has been confirmed by sequence analysis.

[2]When using an internal lane standard, such as the CC5 Internal Lane Standard 500, the calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label and linker also affect migration of alleles.

[3]For a current list of microvariants, see the Variant Allele Report published at the U.S. National Institute of Standards and Technology (NIST) web site at: **www.cstl.nist.gov/div831/strbase/**

[4]Amelogenin is not an STR but displays an 89-base, X-specific band and a 95-base, Y-specific band.

Table 6. The PowerPlex® Fusion System Allele Determinations in Commonly Available Standard DNA Templates.

	Standard DNA Templates[1]		
STR Locus	**2800M**	**9947A**	**9948**
Amelogenin	X, Y	X, X	X, Y
D3S1358	17, 18	14, 15	15, 17
D1S1656	12, 13	18.3, 18.3	14, 17
D2S441	10, 14	10, 14	11, 12
D10S1248	13, 15	13, 15	12, 15
D13S317	9, 11	11, 11	11, 11
Penta E	7, 14	12, 13	11, 11
D16S539	9, 13	11, 12	11, 11
D18S51	16, 18	15, 19	15, 18
D2S1338	22, 25	19, 23	23, 23
CSF1PO	12, 12	10, 12	10, 11
Penta D	12, 13	12, 12	8, 12
TH01	6, 9.3	8, 9.3	6, 9.3
vWA	16, 19	17, 18	17, 17
D21S11	29, 31.2	30, 30	29, 30
D7S820	8, 11	10, 11	11, 11
D5S818	12, 12	11, 11	11, 13
TPOX	11, 11	8, 8	8, 9
DYS391	10	–	10
D8S1179	14, 15	13, 13	12, 13
D12S391	18, 23	18, 20	18, 24
D19S433	13, 14	14, 15	13, 14
FGA	20, 23	23, 24	24, 26
D22S1045	16, 16	11, 14	16, 18

[1]Information on strains 9947A and 9948 is available online at:
http://ccr.coriell.org/Sections/Search/Sample_Detail.aspx?Ref=GM09947 and
http://ccr.coriell.org/Sections/Search/Sample_Detail.aspx?Ref=GM09948
Information about the use of 9947A and 9948 DNA as standard DNA templates can be found in reference 25.

9.B. DNA Extraction and Quantitation Methods and Automation Support

Promega offers a wide variety of reagents and automated methods for sample preparation, DNA purification and DNA quantitation prior to STR amplification.

For analysis of database, reference and other single-source samples, we recommend direct amplification from FTA® punches or preprocessing of swabs and nonFTA punches with the SwabSolution™ Kit or PunchSolution™ Kit. The SwabSolution™ Kit (Cat.# DC8271) contains reagents for rapid DNA preparation from buccal swabs prior to amplification. The procedure lyses cells contained on the swab head and releases into solution sufficient DNA for STR amplification. A small volume of the final swab extract is added to the PowerPlex® reaction. The PunchSolution™ Kit is used to process punches from nonFTA storage cards containing blood or buccal samples prior to direct amplification.

For casework or samples that require DNA purification, we recommend the DNA IQ™ System (Cat.# DC6700), which is a DNA isolation system designed specifically for forensic samples (26). This system uses paramagnetic particles to prepare clean samples for STR analysis easily and efficiently and can be used to extract DNA from stains or liquid samples, such as blood or solutions. The DNA IQ™ Resin eliminates PCR inhibitors and contaminants frequently encountered in casework samples. With DNA-rich samples, the DNA IQ™ System delivers a consistent amount of total DNA. The system has been used to isolate DNA from routine sample types including buccal swabs, stains on FTA® paper and liquid blood. Additionally, DNA has been isolated from casework samples such as tissue, differentially separated sexual assault samples and stains on support materials. The DNA IQ™ System has been tested with PowerPlex® Systems to ensure a streamlined process.

For applications requiring human-specific DNA quantification, the Plexor® HY System (Cat.# DC1000) was developed (27). This qPCR-based method provides total human and male-specific DNA quantification in one reaction. Additionally, the Plexor® HY System provides a post-amplification melt analysis to confirm positive results and and Internal PCR Control (IPC) to confirm negative results. Additional ordering information is available in Section 9.E.

For information about automation of Promega chemistries on automated workstations using Identity Automation™ solutions, contact your local Promega Branch Office or Distributor (contact information available at: **www.promega.com/support/worldwide-contacts/**), e-mail: **genetic@promega.com** or visit: **www.promega.com/idautomation/**

9.C. The CC5 Internal Lane Standard 500

The CC5 Internal Lane Standard 500 contains 21 DNA fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases in length (Figure 24). Each fragment is labeled with CC5 dye and can be detected separately (as a fifth color) in the presence of PowerPlex® Fusion-amplified material. The CC5 ILS 500 is designed for use in each CE injection to increase precision in analyses when using the PowerPlex® Fusion System. Protocols to prepare and use this internal lane standard are provided in Section 5.

Note: Sizing of Penta E and DYS391 alleles ≥475 bases will not use Local Southern Method. For Penta E, alleles >24 will be labeled as "OL."

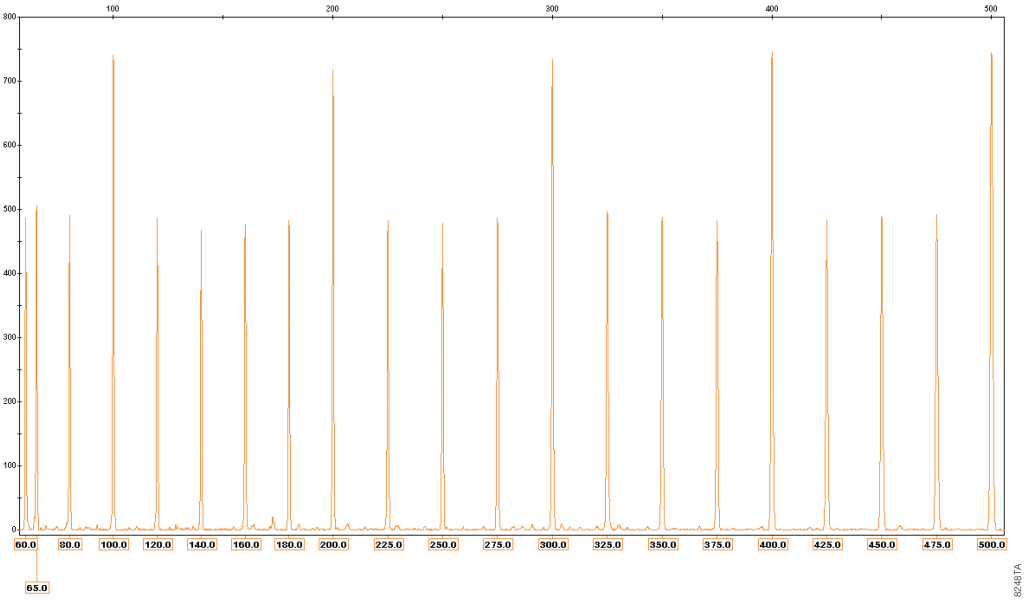

Figure 24. CC5 Internal Lane Standard 500. An electropherogram showing the CC5 Internal Lane Standard 500 fragments.

9.D. Composition of Buffers and Solutions

TE^{-4} Buffer (10mM Tris-HCl, 0.1mM EDTA [pH 8.0])

1.21g	Tris base
0.037g	EDTA ($Na_2EDTA \cdot 2H_2O$)

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 8.0 with HCl. Bring the final volume to 1 liter with deionized water.

TE^{-4} Buffer with 20µg/ml Glycogen

1.21g	Tris base
0.037g	EDTA ($Na_2EDTA \cdot 2H_2O$)
20µg/ml	glycogen

Dissolve Tris base and EDTA in 900ml of deionized water. Adjust to pH 8.0 with HCl. Add glycogen. Bring the final volume to 1 liter with deionized water.

9.E. Related Products

STR Systems

Product	Size	Cat.#
PowerPlex® Y23 System	50 reactions	DC2305
	200 reactions	DC2320
PowerPlex® 21 System	200 reactions	DC8902
	4 × 200 reactions	DC8942
PowerPlex® 18D System	200 reactions	DC1802
	800 reactions	DC1808
PowerPlex® ESX 16 System	100 reactions	DC6711
	400 reactions	DC6710
PowerPlex® ESX 17 System	100 reactions	DC6721
	400 reactions	DC6720
PowerPlex® ESI 16 System	100 reactions	DC6771
	400 reactions	DC6770
PowerPlex® ESI 17 Pro System	100 reactions	DC7781
	400 reactions	DC7780
PowerPlex® 16 HS System	100 reactions	DC2101
	400 reactions	DC2100
PowerPlex® CS7 System	100 reactions	DC6613

Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
PowerPlex® 5-Dye Matrix Standards, 3100/3130*	25µl (each dye)	DG4700
PunchSolution™ Kit*	100 preparations	DC9271
SwabSolution™ Kit*	100 preparations	DC8271
CC5 Internal Lane Standard 500	300µl	DG1521
2800M Control DNA (10ng/µl)*	25µl	DD7101
2800M Control DNA (0.25ng/µl)*	500µl	DD7251
Water, Amplification Grade	6,250µl (5 × 1,250µl)	DW0991

*Not for Medical Diagnostic Use.

9.E. Related Products (continued)

Sample Preparation and DNA Quantification Systems

Product	Size	Cat.#
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700
Plexor® HY System*	200 reactions	DC1001
	800 reactions	DC1000

*Not for Medical Diagnostic Use.

9.F. Summary of Changes

The following change was made to the 5/14 revision of this document:

Legal disclaimers were updated.

[a]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

[b]U.S. Pat. Nos. 5,843,660, 6,479,235, 6,221,598 and 7,008,771, Australian Pat. No. 724531, Canadian Pat. No. 2,118,048 and 2,251,793, Korean Pat. No. 290332, Singapore Pat. No. 57050, Japanese Pat. Nos. 3602142 and 4034293, Chinese Pat. Nos. ZL99813729.4 and ZL97194967.0, European Pat. No. 0960207 and other patents pending.

[c]U.S. Pat. No 6,238,863, European Pat. No. 1058727, Chinese Pat. No. ZL99802696.4, Japanese Pat. No. 4494630 and other patents pending.

[d]STR loci are the subject of U.S. Pat. No. RE 37,984, German Pat. No. DE 38 34 636 C2 and other patents issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, e.V., Germany.

[e]Allele sequences for one or more of the loci vWA, FGA, D8S1179, D21S11 and D18S51 in allelic ladder mixtures is licensed under U.S. Pat. Nos. 7,087,380, 7,645,580, Australia Pat. No. 2003200444 and corresponding patent claims outside the US.

[f]TMR-ET, CXR-ET and CC5 dyes are proprietary.

(g)This product or portions thereof is manufactured and sold under license from GE Healthcare under Australia Pat. No. 692230, Austria Pat. No. E236994, Belgium Pat. No. 0743987, Canada Pat. No. 2231475, EP Pat. Nos. 0743987 and 0851867, France Pat. Nos. 0743987 and 0851867, Germany Pat. Nos. 19581489, 69530286.8 and 0851867, Italy Pat. Nos. 0743987 and 0851867, Japan Pat. No. 3066984, Liechtenstein Pat. Nos. 0743987 and 0851867, Netherlands Pat. Nos. 0743987 and 0851867, Spain Pat. Nos. 2197193 and 2173310, Sweden Pat. Nos. 0743987 and 0851867, Switzerland Pat. Nos. 0743987 and 0851867, United Kingdom Pat. Nos. 0743987 and 0851867, U.S. Pat. Nos. 5,654,419, 5,688,648, 5,869,255, 6,177,247, 5,707,804, 6,028,190, 6,544,744, 7,015,000 and 5,728,528 and other pending and foreign patent applications.

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